JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

ORIGINAL ARTICLE | DOI: 10.5584/jiomics.v10i1.307

S-glutathionylation of proteins in various types of neurodegenerative pathology and protective effects of pantothenic acid derivatives

D. S. Semenovich[1], N. P. Kanunnikova[2]

[1] Institute of Biochemistry of Biologically Active Substances, National Academy of Science of Belarus, BLK, 50, Grodno, 230017, Belarus; [2] Yanka Kupala State University of Grodno, 22 Ozheshko Street, Grodno, 230023, Belarus

Received: 3 November 2019 **Accepted:** 01 March 2020 **Available Online**: 31 March 2020

Abstract

We studied changes in S-glutathionylated proteins (PSSG) content in rat brain structures *in vivo* after administration of Fe+LPS, aluminum chloride, rotenone, and 3-nitropropionic acid, and in vitro experiments with oxidative stress on rat brain tissue culture or on subcellular fractions, as well as possibility of correcting these changes with pantothenic acid precursors – panthenol, pantethine and homopantothenic acid (HPA). We have shown that the content of PSSG significantly increases in brain structures in all the models of neurotoxicosis, and this increase is observed to the greatest extent precisely in those structures (hemispheres, hippocampus, basal ganglia) where a particular neurotoxin has the most pronounced effect. Thus, the protein glutathionylation can initiate dysfunction of proteins and contribute to the development of neurodegeneration. Precursors of CoA reduce S-glutathionylation of proteins, since HPA, which is not a precursor of CoA, does not have a protective effect in relation to PSSG.

Аннотация

Были изучены изменения в содержании S-глутатионилированных белков (PSSG) в структурах мозга крыс *in vivo* после введения Fe + ЛПС, хлорида алюминия, ротенона и 3-нитропропионовой кислоты, а также в экспериментах in vitro при окислительном стрессе на культуре ткани мозга крысы или на субклеточных фракциях, а также возможность коррекции этих изменений с помощью предшественников пантотеновой кислоты – пантенола (ПЛ), пантетина (ПТ) и гомопантотеновой кислоты (ГПК). Установлено, что содержание PSSG значительно увеличивается в структурах головного мозга во всех моделях нейротоксикоза, и это увеличение наблюдается в наибольшей степени именно в тех структурах (больших полушариях, гиппокампе, базальных ганглиях), где данный нейротоксин проявляет наиболее выраженное действие. Очевидно, процесс S-глутатионилирования белков может инициировать нарушения их функций и способствовать развитию нейродегенерации. Предшественники биосинтеза КоА снижают S-глутатионилирование белков, тогда как ГПК, который не является предшественником КоА, не оказывает защитного действия на этот показатель.

Keywords: Protein S-glutathionylation, neurodegenerative pathology, neurotoxicosis, brain structures, panthothenic acid derivates.

1. Introduction

Currently known, disorders in brain structures in such neurodegenerative pathologies as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), etc., are accompanied by the development of oxidative stress [1, 2]. In the mechanisms that ensure the maintenance of redox balance in the brain tissue, the glutathione system plays an important role [3, 4].

The levels of GSH were proposed to use the determination of the GSH level as a marker of moderate kognitive impairment in AD [5].

In addition to participating in redox reactions, glutathione is involved in protein glutathionylation reactions. Protein S-glutathionylation is a specific oxidative post-translational modification characterized by the reversible formation of a mixed disulfide bond between Cys protein residues and glutathione [6]. The high level of GSH in the cells and the easy conversion of sulfenic acids and S-nitro derivatives to

***Corresponding author:** Semenovich Dmitry, semenovich@ibiochemistry.by, tel: +375 29 854-35-12

glutathione-mixed disulfides suggest that reversible glutathionylation may be a common mechanism of redox signal transmission and regulation of the activity of redox-sensitive thiol-containing proteins [7]. Currently, over 100 proteins have been identified, the activity of which changes as a result of post-translational S-glutathionylation [6]. For example, as a result of S-glutathionylation, the activity of apoptosis regulation proteins (caspase-3), folding and degradation proteins (heat shock protein HSP70), energy metabolism proteins (glyceraldehyde-3-phosphate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase complex) significantly changes [8, 9, 10].

Glutathionylation can occur through non-enzymatic or enzymatic reactions. Enzymes that catalyze the reaction of formation of S-glutathionylated proteins (PSSG) are thioltransferases - glutathione-S-transferase P, glutathione-S -transferase O1 of the omega class. The non-enzymatic formation of S-glutathionylated proteins (PSSG) depends on the availability of GSH and / or GSSG. PSSG are easily and reversibly formed via GSH binding by Cys residues in target proteins under the action of glutaredoxins and thioredoxins [4, 13].

S-glutathionylation proceeds both under normal physiological conditions when the GSH / GSSG ratio in the brain is about 100: 1 [14], and in conditions of changes of redox balance in oxidative stress, when the GSH / GSSG ratio can significantly decrease. S-glutathionylation, on the one hand, is a protective mechanism against the action of reactive oxygen species, and, on the other hand, by changing the activity of certain enzymes, it can lead to changes in energy metabolism, folding and protein degradation, and regulation of apoptosis, which play an important role in the pathogenesis of neurodegenerative disorders [1–3, 6, 7]. Recent studies have shown that glutathionylation of specific proteins can contribute to the onset or progression of AD, PD, HD and other neurodegenerative diseases [9-12, 15].

Based on this, we studied changes in the PSSG content in rat brain structures in different experimental models of neurodegenerative diseases, which are characterized by different mechanisms for the development of pathological changes in brain tissue. What was common to them was that in all cases the development of oxidative stress was observed and, accordingly, a shift in the redox balance and changes in the glutathione system [3, 4]. We also studied the possibility of correcting these changes with pantothenic acid derivatives. Earlier, we found the presence of pronounced neuroprotective activity in CoA precursors, carried out through interaction with the glutathione system [16].

2. Material and Methods

Experimental models were performed on male Wistar CRL: (WI) WUBR rats weighing 180–200 g, kept under standard vivarium conditions. All experiments with laboratory animals were carried out in accordance with ethical standards, as well as the rules for conducting scientific work using experimental animals in scientific research, compiled on the basis of recommendations and requirements of the World Animal Welfare Society (WSPA) and the European Convention for the Protection of Experimental Animals (Strasbourg, 1986).

Derivatives of pantothenic acid (PA) D-panthenol (PL) and D-pantethine (PT), which are the precursors of CoA biosynthesis, as well as homopantothenic acid (HPA, hopantene), which is unable to convert to CoA, were used as modulators of metabolic disturbances during oxidative stress. These drugs were administered at a dose of 200 mg / kg, intragastrically.

Administration of iron (II) gluconate and E. coli lipopolysaccharide (LPS) to model PKAN (pantothenate kinase-associated neurodegeneration) [17, 18]. 10–12-Day-old rats weighing 20 ± 5 g were injected with iron (II) gluconate (30 mg / kg, intragastrically) for 20 days. From 21 days, PA derivatives were administered for 14 days. The day before decapitation, E. coli lipopolysaccharide (200 μg / kg, ip) was administered.

Administration of aluminum chloride for modeling AD [19]. Aluminum chloride (200 mg / kg, intragastrically) was administered daily to rats for 6 weeks. From the 5th week of the experiment, PA derivatives were administered daily for 14 days.

Administration of rotenone for modeling PD [20, 21]. Rats were injected with rotenone daily for 6 weeks (2.5 mg / kg, subcutaneously, diluent — a mixture of DMSO with sunflower oil). From the 5th week of the experiment, PA derivatives were administered daily for 14 days.

Administration of 3-nitropropionic acid for modeling HD [22]. Rats were administered 3-nitropropionic acid daily for 14 days (NPA, 10 mg / kg, intraperitoneally). PA derivatives were administered daily for 14 days, too.

Brain structures examined. We studied the level of PSSG in the cerebral hemispheres, hippocampus, basal ganglia, brain stem and cerebellum isolated from rat brain, as well as in brain cell culture from rat embryos.

Primary cell culture and isolation subcellular fractions from brain tissue.

The primary brain cell culture of 18-day-old rat embryos was isolated using mechanical and enzymatic tissue disaggregation with trypsin [23, 24]. Cells were incubated in Eagle's medium (MEM) containing 5% thermally inactivated rat serum, 5.55 mM glucose, 2 mM glutamine, 20 U / ml penicillin and 20 U / ml streptomycin in 50 mm diameter plastic Petri dishes pretreated poly-D / L-lysine [23, 24]. The cell culture was incubated at 37 ° C in a gas mixture containing 5% CO2. Cell viability in culture was assessed by the release of lactate dehydrogenase into the extracellular medium.

In the another experiment subcellular fractions were isolated from rat cerebral tissue by differential centrifugation using a selection medium containing 0.32 M sucrose, 10 mM Tris HCl, pH 7.4 and 1 mM EDTA. Mitochondrial sediment was resuspended in isolation medium at the rate of 0.4 ml of

Table 1 | The effect of PL and PT on the content of PSSG (μmol / g tissue) in the brain structures after the administration of iron (II) gluconate and LPS, (M ± SEM, n = 8). Влияние ПЛ и ПТ на содержание PSSG (мкмоль / г ткани) в структурах мозга крыс после введения глюконата железа (II) и ЛПС (M±SEM, n=8). Notes: * - p <0.05 as compared to the control group, # - p <0.05 as compared to the Fe+LPS group.

Groups	Hemispheres	Basal ganglia	Hippocampus
Control	0.1 ± 0.1	0.1 ± 0.03	0.11 ± 0.04
Fe+LPS	0.13 ± 0.03*	0.11 ± 0.01*	0.12 ± 0.01*
Fe+LPS+PL	0.13 ± 0.01*	0.08 ± 0.02*#	0.11 ± 0.02#
Fe+LPS+PT	0.12 ± 0.01*#	0.11 ± 0.01*	0.09 ± 0.03*#

medium per mitochondrial precipitate isolated from 1 g of tissue. Cells and mitochondria were destroyed using a lysis buffer containing 20 mM Tris HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.2% Triton X-100.

The content of PSSG was determined by spectrofluorimetric method using 2,3-naphthalenedicarboxaldehyde (NDA) by the technique of Menon, Board [25] with our modifications. In order to avoid formation of free thiols (predominantly GSH) in the samples, they are blocked by the inclusion of 10 mM NEM (N-ethylmaleimide) in the homogenizing solution. After centrifugation a tissue extract was precipitated with twice the volume of ice-cold acetone to remove the alkylated cellular free thiols and excess NEM. After second centrifugation, the supernatant was discarded and the precipitated proteins were washed again in ice-cold acetone and allowed to air dry. The pellet was suspended in 0.5 M Tris·HCl, pH 8.2, containing 0.1% Triton X-100.

The reducing agent tris(2-carboxyethyl)phosphine (TCEP) was added to the protein solution (final concentration of 5 mM) to break the disulfide protein bonds and elute the protein-bound GSH. The deglutathionylated proteins was then precipitated by the addition 5-sulfosalicylic acid on ice for 30 min, and the precipitated proteins were removed by centrifugation. Releasing GSH from proteins in the supernatant was detected with NDA spectrofluorimetrically (Ex=485 nm, Em =520 nm). The results were calculated as nmol GSH/mg protein by reference to a standard curve generated with GSH.

Total protein was determined by the Bradford method [26].

Statistical processing of experimental data was performed using Microsoft Excel 2016, GraphPad Prism 6.0. The experimental data were presented as M ± SEM, where M is the average value, SEM is the standard error of the mean. The significance of intergroup differences was evaluated using one-way analysis of variance (ANOVA) using the Tukey test. In all cases, differences were considered statistically significant at a value of p <0.05.

3. Results

We have studied changes in the content of PSSG in rat brain structures in different models of neurodegeneration.

Since iron does not penetrate well through the blood-brain barrier in adult animals, we administered iron preparations to 10–12-day-old rat pups when the barrier permeability is still high [17, 18]. This contributes to the accumulation of iron in the brain tissue, and mainly in the striatum of the cerebral hemispheres. An additional single administration of LPS to animals on the 34th day of their life leads to the development of inflammation and can serve as an experimental model of PKAN, which is characterized by the accumulation of iron and due to impaired CoA biosynthesis due to a genetic defect in the key enzyme of its biosynthesis – pantothenate kinase [27]. The control group consisted of animals of the same age, which were injected with a solution of sodium chloride 0.85 %.

We found that the content of PSSG after administration of iron (II) gluconate and LPS in the cerebral hemispheres increased by 26%, in basal ganglia by 11% and in the hippocampus by 14% (table 1).

Although PL did not affect the process of S-glutathionylation of proteins in the cerebral hemispheres, it was shown that it decreased S-glutathionylated proteins in the hippocampus to the control level, and even lower than the values in the control in basal ganglia (p <0.05). Against this background, PL did not affect the process of S-glutathionylation of proteins in the cerebral hemispheres, reduced their content in the hippocampus to the control level, and in basal ganglia reduced their content even lower than the values in the control (p<0.05). Conversely, tThe presence of PT did not affect this indicator of basal ganglia, decreased the level of PSSG in hemispheres, and in hippocampus decreased it below the control values.

To model AD symptoms in rats, we used a model with intragastrically administeredration of aluminum chloride for 6 weeks. This led to the development of neurotoxicosis in animals, accompanied by disturbances in the redox balance and pronounced changes in the redox potential of the glutathione system in brain tissue [28].

The action of aluminum chloride led to a significant increase in the content of PSSG in all the rat brain structures we studied, which, obviously, is an indicator of an increase in the post-translational modification of proteins under conditions of a shift in the thiol disulfide balance (table 2). It can be noted that the level of PSSG changes the least in the brain stem. The introduction of all PA derivatives (PL, PT, and HPA) led to the return of restored the PSSG content in hemispheres and hippocampus to the control level, and even lower than the control values in other brain structures.

Together, these results indicate Thus, that the process of S -glutathionylation of proteins under the action of aluminum chloride is activated in all brain structures of rats, and PA derivatives are effective correctors that reduce post-translational modification of proteins under conditions of

Table 2 | The effect of PL, PT, and HPA on the content of PSSG (nmol / mg protein) in the brain structures after the administration of AlCl3 (M±SEM, n=8). Влияние ПЛ, ПТ и ГПК на содержание PSSG (нмоль / мг белка) в структурах мозга крыс после введения AlCl3 (M±SEM, n=8). Notes: * - p <0.05 as compared to the control group, # - p <0.05 as compared to the AlCl3 group

Groups	Hemispheres	Basal ganglia	Hippocampus	Brain stem	Cerebellum
Control	0.47 ± 0.04	0.52 ± 0.04	0.25 ± 0.04	0.28 ± 0.03	0.24 ± 0.03
$AlCl_3$	0.60 ± 0.05*	0.58 ± 0.02*	0.28 ± 0.05*	0.31 ± 0.05*	0.29 ± 0.05*
$AlCl_3$ +PL	0.46 ± 0.08#	0.48 ± 0.05#	0.26 ± 0.04#	0.27 ± 0.03#	0.23 ± 0.03#
$AlCl_3$ + PT	0.47 ± 0.06#	0.40 ± 0.06*#	0.24 ± 0.02#	0.26 ± 0.02#	0.21 ± 0.02#
$AlCl_3$ + HPA	0.34 ± 0.02*#	0.51 ± 0.06#	0.25 ± 0.06#	0.24 ± 0.04#	0.24 ± 0.04#

oxidative stress in the central nervous system.

Rotenone is one of the neurotoxins used to model PD *in vitro* and *in vivo* [21]. Administration of rotenone to animals causes biochemical, histological and behavioral symptoms similar to those observed in patients with PD. Rotenone is an inhibitor of the mitochondrial I complex I of the electron transport chain of mitochondria, resulting in the formation of free radicals and the development of oxidative stress [229]. In our experiments, it was found that the effect of rotenone was accompanied by an increase in the content of PSSG in the cerebral hemispheres by 20%, the hippocampus by 18% and, most notably, in the basal ganglia by 56 % especially pronounced (by 56%) in the basal ganglia (table 3). Predictably, rotenone is known to cause the most prominent damage to neurones in the basal ganglia. It is well known, that in this structure of the brain rotenone causes the most pronounced damage to neurons.

PL and PT contributed to the weakening of the effect of rotenone on this indicator in the cerebral hemispheres and the hippocampus, returning it to the values in the control, while in basal ganglia the effect of their exposure was insufficient to return the PSSG level to normal. HPA had no effect on the content of PSSG against rotenone.

Table 3 | The effect of PL, PT, and HPA on the content of PSSG (nmol / mg protein) in the brain structures after the administration of rotenone (M±SEM, n=8). Влияние ПЛ, ПТ и ГПК на содержание PSSG (нмоль / мг белка) нмоль / мг белка) в структурах мозга крыс после введения ротенона (M±SEM, n=8). Notes: * - p <0.05 as compared to the control group, # - p <0.05 as compared to the rotenone group .

Groups	Hemispheres	Basal ganglia	Hippocampus
Control	0.50 ± 0.02	0.57 ± 0.02	0.51 ± 0.03
Rotenone	0.59 ± 0.02*	0.88 ± 0.03*	0.60 ± 0.02*
Rotenone	0.52 ± 0.02#	0.72 ± 0.02*#	0.52 ± 0.01#
Rotenone	0.51 ± 0.02#	0.69 ± 0.02*#	0.51 ± 0.01#
Rotenone	0.53 ± 0.01*	0.90 ± 0.03*#	0.61 ± 0.02*

NPA is a mitochondrial toxin that causes selective degeneration of neurons in the striatum and the development of symptoms characteristic of HD in experimental animals [2930]. Oxidative stress is one of the important factors in the pathogenesis of HD [3031]. In this model, we also observed an increase in the PSSG content in hemispheres by 20% and hippocampus by 23%, while in basal ganglia this increased by 65% (table 4).

PL and PT also returned the PSSG level to control in hemispheres and hippocampus, while in basal ganglia they contributed only to its slight decrease relative to the value against the background of NPA. The effect of HPA was weaker in all studied brain structures.

In order to clarify the mechanisms of the protective effect of PA derivatives on protein glutathionylation processes, we studied changes in this parameter in *in vitro* models of oxidative stress. For this, we used the primary brain cell culture of 18-day-old rat embryos. Derivatives of PA with final concentrations of 10, 25, 50, 100, and 500 μM were added to the cell suspension (0.5–1.0 mg / ml total protein) and preincubation was performed for 30 min at 37 ° C. To induce oxidative stress, 50 μM tBHP was added and samples were incubated for 30 min at 37 ° C. It was shown that the development of oxidative stress initiated by tert-butyl hydroperoxide (tBHP) was accompanied by an almost 3-fold increase in the content of PSSG in the culture of brain cells of rat embryos (figure). The introduction of both PL and PT

Table 4 | The effect of PL, PT, and HPA on the content of PSSG (nmol / mg protein) in the brain structures after the administration of NPA (M±SEM, n=8). Влияние ПЛ, ПТ и ГПК на содержание PSSG (нмоль / мг белка) в структурах мозга крыс после введения 3-нитропропионовой кислоты (M±SEM, n=8). Notes: * - p <0.05 as compared to the control group, # - p <0.05 as compared to the NPA group.

Groups	Hemispheres	Basal ganglia	Hippocampus
Control	0.48 ± 0.01	0.57 ± 0.01	0.53 ± 0.03
NPA	0.58 ± 0.01*	0.93 ± 0.01*	0.65 ± 0.02*
NPA+PL	0.52 ± 0.01#	0.71 ± 0.01*#	0.55 ± 0.01#
NPA+PT	0.53 ± 0.01#	0.69 ± 0.01*#	0.57 ± 0.01#
NPA+HPA	0.51 ± 0.01*	0.83 ± 0.02*#	0.60 ± 0.02*

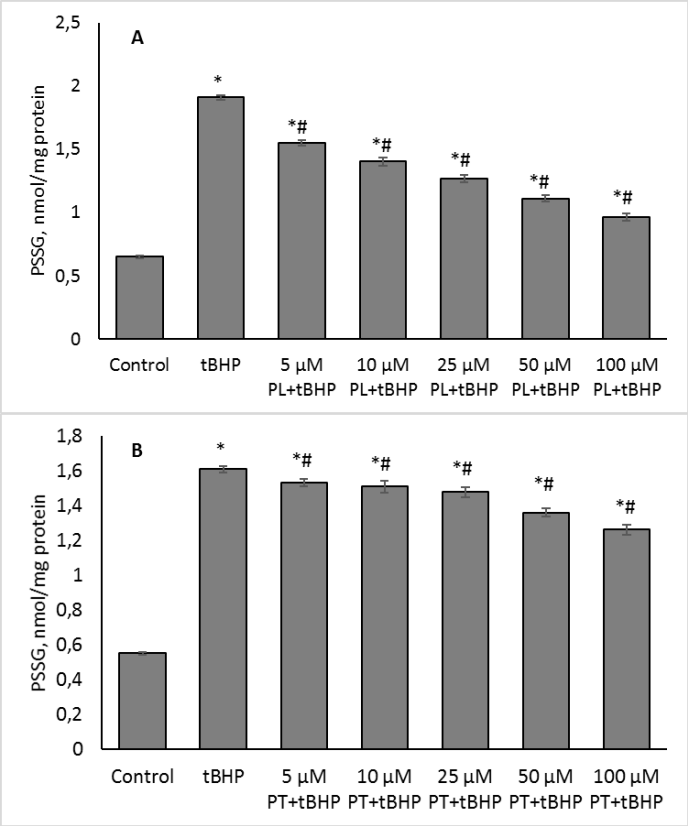

Figure 1 | Influence of tBHP (50 μM), PL (histogram A) and PT (histogram B) on the level of PSSG in the primary brain cell culture of 18-day rat embryos. Влияние tBHP (50 μM), ПЛ (гистограмма А) и ПТ (гистограмма В) на содержание PSSG в первичной культуре клеток головного мозга 18-дневных эмбрионов крыс. Notes: * - p <0.05 as compared to the control group, # - p <0.05 as compared to the tBHP group.

into the incubation medium in a dose-dependent manner contributed to a decrease in the content of PSSG, which indicates a direct effect of the drugs on the processes of glutathionylation of proteins.

In the next experiment, we examined in which subcellular fraction the proteins undergo had the greatest modification of proteins. For this, PL or PT with the final concentrations of 10, 25, 50, 100, and 500 μM were added to the homogenate of brain tissue or a suspension of subcellular fractions (1.5–2 mg / ml of total protein) and preincubated for 30 min at 37 ° C. 100 μM tBHP was added into the samples to induce oxidative stress and incubated during 15 min in 37 °C.

It was established that, against the background of tBHP, the content of PSSG increased both in the total homogenate and in mitochondria, microsomes, and in the cytosol (table 5). In the presence of PL in the incubation medium, the effect of tBHP was not manifested, and the level of PSSG did not differ from the values in the control group either in the total homogenate or in the subcellular fractions studied. However, in the nuclear fraction, the content of S-glutathionylated proteins did not change either against tBHP or in the presence of PL.

4. Concluding Remarks

Protein S-glutathionylation has the ability to regulate a number of biological functions of proteins in the cell, both in normal and pathological conditions. It is generally accepted that S-glutathionylation of proteins is considered one of the mechanisms of protein protection against the oxidative effects of reactive oxygen species [1–3, 6]. We have shown that under the conditions of the development of oxidative stress, the content of PSSG significantly increases in brain structures in all the models of neurotoxicosis that we studied, and this increase is observed to the greatest extent precisely in those structures where a particular neurotoxin has the most pronounced effect. As a rule, with neurodegenerative diseases, pathological changes occur primarily in the cortical and subcortical structures of the hemispheres [32, 33, 34]. According to our data, changes in PSSG levels in the brain stem were minimal in the models of experimental neurodegeneration.

PA derivatives, precursors of CoA, reduce S-glutathionylation of proteins, which can be regarded as a protective effect against protein conformation disorders and preservation of their biological activity during oxidative stress, and these effects are obviously mediated through the CoA system, since HPA, which is not a precursor of CoA, does not have a protective effect in relation to PSSG.

Thus, the activation of S-glutathionylation of proteins is a characteristic sign of metabolic disorders of redox balace in brain tissue during oxidative stress and is observed in different ways of modeling neurodegenerative processes. Given the fact that glutathionylation of proteins can also initiate misfolding of proteins and their aggregation into insoluble complexes, impaired mitochondrial functions, iron accumulation in brain structures, and contribute to the progression of death of neurons, therefore it can be assumed that glutathionylation of proteins can not only protect them from irreversible damage by free radicals during oxidative

Table 5 | The effect of PL (100 μM) on the content of PSSG (nmol / mg protein) in the subcellular fractions of hemispheres in the presence of tBHP (100 μM) (M±SEM, n=4). Влияние ПЛ (100 μM) на содержание PSSG (нмоль / мг белка) в субклеточных фракциях больших полушарий мозга в присутствии tBHP (100 μM) (M±SEM, n=4). Notes: * - p <0.05 as compared to the control group, # - p <0.05 as compared to the tBHP group.

Subcellular	Control	tBHP	tBHP + 0.1
Homogenate 1:10 (w/v)	1.73 ± 0.01	2.31 ± 0.03*	1.81 ± 0.02#
Nucleus	0.14 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
Mitochondria	0.55 ± 0.02	0.92 ± 0.02*	0.63 ± 0.01#
Microsomes	0.28 ± 0.01	0.39 ± 0.01*	0.31 ± 0.02#
Cytosol	0.76 ± 0.02	0.88 ± 0.02*	0.76 ± 0.02#

stress, but also perform certain regulatory functions in the cell, having a close relationship with to CoAlation functions. Recently it has been shown that protein CoAlation is a reversible post-translational modification induced by oxidizing agents and metabolic stress in prokaryotic and eukaryotic cells [335]. During physiological conditions CoA produces metabolically-active derivatives, but may act as an antioxidant in response to oxidative or metabolic stress. The close relationship between CoA-lation and S-glutathionylation is evidenced by the pronounced effects of CoA biosynthesis precursors - PL and PT, but not HPA, on the level of S-glutathionylated proteins in brain structures in the experimental models of neurotoxicosis. It must be taken into account when considering the mechanisms of the pathogenesis of neurodegenerative diseases and may be the rationale for the use of pantothenic acid derivatives for treatment of neurodegenerative disorders.

Заключение

S-глутатионилирование белков обладает способностью регулировать ряд биологических функций белков в клетке как при нормальных, так и при патологических состояниях. Известно, что S-глутатионилирование белков считается одним из механизмов защиты белков от окислительного воздействия активных форм кислорода. Мы показали, что в условиях развития окислительного стресса содержание PSSG значительно возрастает в структурах головного мозга во всех изученных нами моделях нейротоксикоза, и это увеличение наблюдается в наибольшей степени именно в тех структурах, где конкретный нейротоксин обладает наиболее выраженным действием. Как правило, при нейродегенеративных заболеваниях патологические изменения происходят в первую очередь в корковых и подкорковых структурах больших полушарий мозга. В изученных нами моделях экспериментальной нейродегенерации изменения уровня PSSG в стволе головного мозга были наименее выраженными.

Производные пантотеновой кислоты, предшественники КоА, снижают S-глутатионилирование белков, что можно рассматривать как защитный эффект против нарушений конформации белка и сохранение их биологической активности во время окислительного стресса, и эти эффекты, очевидно, опосредуются через систему КоА, поскольку гомопантотеновая кислота, которая не является предшественником КоА, не оказывает защитного действия на этот показатель.

Таким образом, активация S-глутатионилирования белков является характерным признаком нарушений окислительно-восстановительного баланса в ткани мозга при окислительном стрессе и наблюдается при разных способах моделирования нейродегенеративных процессов. Учитывая тот факт, что глутатионилирование белков также может инициировать нарушения сворачивания белков и их агрегацию в нерастворимые комплексы, нарушение функций митохондрий, накопление железа в структурах головного мозга и способствовать прогрессированию гибели нейронов, можно полагать, что глутатионилирование белков может не только защищать их от необратимого повреждения свободными радикалами во время окислительного стресса, но также выполнять определенные регуляторные функции в клетке, тесно связанные с функциями КоА-лирования. Недавно было показано, что КоА-лирование белка является обратимой посттрансляционной модификацией, вызванной окислителями и метаболическим стрессом в прокариотических и эукариотических клетках. В физиологических условиях КоА способствует образованию метаболически активных производных, но он может также действовать как антиоксидант в ответ на окислительный или метаболический стресс. О тесной взаимосвязи между КоА-лированием и S-глутатионилированием свидетельствуют выраженные эффекты предшественников биосинтеза КоА – ПЛ и ПТ, но не ГПК, на уровень S-глутатионилированных белков в структурах головного мозга в экспериментальных моделях нейротоксикоза. Эти факты необходимо учитывать при рассмотрении механизмов патогенеза нейродегенеративных заболеваний и могут служить обоснованием использования производных пантотеновой кислоты для лечения нейродегенеративных заболеваний.

References

[1] L. M. Sayre, G. Perry, M. A. Smith. Chemical Research in Toxicology. 21 (2008) 172–188

[2] J. Li, O. Wuliji, W. Li, Z. G. Jiang, H. A. Ghanbari. International Journal of Molecular Sciences. 14 (2013) 24438 –24475

[3] W. M. Johnson, A. L. Wilson-Delfosse, J. J. Mieyal. Nutrients. 4 (2012) 1399–1440

[4] G. J. McBean, M. Aslan, H. R. Griffiths, R. C. Torrão. Redox Biology. 5 (2015) 186–194

[5] P. K. Mandal, S. Saharan, M. Tripathi, G. Murari. Biological Psychiatry. 78 (2015)

[6] D. Popov. Archives of Physiology and Biochemistry. 120 (2014) 123–130

[7] I. Dalle-Donne, G. Colombo, N. Gagliano, R. Colombo, D. Giustarini, R. Rossi, A. Milzani. Free Radical Research. 45 (2010) 3–15

[8] A. Pastore, F. Piemonte. European Journal of Pharmaceutical Sciences. 46 (2012) 279–292

[9] A. Young, R. Gill, R. J. Mailloux. Chem. Biol. Int. 299 (2019) 151–162

[10] F. Di Domenico, G. Cenini, R. Sultana, M. Perluigi, D. Uberti, M. Memo, D. A. Butterfield. Neurochemical Research. 34 (2009) 727–733

[11] S. F. Newman, R. Sultana, M. Perluigi, R. Coccia, J. Cai, W. M. Pierce, J. B. Klein, D. M. Turner, D. A. Butterfield. Journal of Neuroscience Research. 85 (2007) 1506–1514

[12] C. Hong, H. Seo, M. Kwak, J. Jeon, J. Jang, E. M. Jeong, J. Myeong, Y. J. Hwang, K. Ha, M. J. Kang, K. P. Lee, E. C. Yi, I. G. Kim, J. H. Jeon, H. Ryu, I. So. Brain. 138 (2015) 3030–3047
[13] M. M. Gallogly, J. J. Mieyal. Current Opinion in Pharmacology. 7 (2007) 381–391
[14] D. Giustarini, I. Dalle-Donne, A. Milzani, R. Rossi. Mechanisms of Ageing and Development. 132 (2011) 141–148
[15] S. J. Cha, H. Kim, H. J. Choi, S. Lee, K. Kim. Oxidative Medicine and Cellular Longevity. 2017 (2017) 1–9
[16] M. Y. Stepanichev, D. A. Markov, S. V. Freiman, O. A. Frolova, S. N. Omelyanchik, T. A. Borodina, N. P. Kanunnikova, A. G. Moiseenok, N. V. Gulyaeva. Neurochemical Journal. 10 (2016) 120–130
[17] M. N. de Lima, M. Polydoro, D. C. Laranja, F. Bonatto, E. Bromberg, J. C. Moreira, F. Dal-Pizzol, N. Schröder. European Journal of Neuroscience. 21 (2005) 2521–2528
[18] N. Schröder, L. S. Figueiredo, M. N. de Lima. Journal of Alzheimer's Disease 34 (2013) 797–812
[19] A. Prakash, A. Kumar. Basic & Clinical Pharmacology & Toxicology. 105 (2009) 98–104
[20] E. Haque, H. Javed, S. Azimullah, S. B. Abul Khair, S. Ojha. Drug Design, Development and Therapy. (2015) 5499
[21] Z. N. Zhang, J. S. Zhang, J. Xiang, Z. H. Yu, W. Zhang, M. Cai, X. T. Li, T. Wu, W. W. Li, D. F. Cai. Brain Research. 1655 (2017) 104–113
[22] P. Kumar, H. Kalonia, A. Kumar. Journal of Asian Natural Products Research. 11 (2009), 439–450
[23] I. L. Salazar, M. Mele, M. Caldeira, R. O. Costa, B. Correia, S. Frisari, C. B. Duarte. Bio-protocol. 7 (2017) e2551
[24] M. A. Dichter. Brain Research 149. (1978) 279–293
[25] D. Menon, P. G. Board. Analytical Biochemistry. 433 (2013) 132–136
[26] M. Bradford. Analytical Biochemistry. 72 (1976) 248–254
[27] S. Jackowski. Journal of Experimental Neuroscience. 13 (2019)
[28] N. Xiong, X. Long, J. Xiong, M. Jia, C. Chen, J. Huang, D. Ghoorah, X. Kong, Z. Lin, T. Wang. Critical Reviews in Toxicology. 42 (2012) 613–632
[29] P. Kumar, P. Ssv, N. Psand, A. Kumar. Cell and Tissue Research. 7 (2007) 853–860
[30] A. Kumar, R. R. Ratan. Journal of Huntington's Disease. 5 (2016) 217–237
[31] I. Gout. Biochemical Society Transactions. 46 (2018) 721–728
[32] T. l. Perry et al. Ann. Neurol. 18 (1985) P.482–489.
[33] A. D. Gitler, A. Chesi et al. Nat. Genet. (41) (2009) 308-315.
[34] A.G. Moiseenok, S.N. Omelyanchik, A.A.Sheval et al. Neurochem. J. 4(1) (2010).30–34.
[35] I. Gout, Biochemical Society Transactions 46(3) (2018) 721 –728.