Effect of Drinking Ration with Reduced Deuterium Content on Brain Tissue Prooxidant-Antioxidant Balance in Rats with Acute Hypoxia Model

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Abstract: The aim was to investigate prooxidant-antioxidant system in the blood and brain homogenates functional activity in rats with acute hypoxia model with different deuterium/protium (D/H) ratios in drinking diet. Studies have shown that consuming DDW (–665 ‰) within 8 weeks lead to deuterium concentration decrease in blood plasma at 317 ‰ and brain at 209 ‰ of rats, in comparison to control group, consuming natural water. DDW consumption before hypoxia modeling in rats improves antioxidant defense enzymes (catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase) activity in the blood, increasing its antioxidant potential by 20 %, while free radical oxidation intensity in plasma and biomolecules peroxide modification rate in erythrocytes. Also, in brain tissues consuming DDW, there were no abnormalities in catalase, superoxide dismutase activity, and it was noted increase (by 71 %) in reduced thiol-containing compounds concentration, which reduces nerve hypoxic cell damage risk. Neuroprotective effect presence is confirmed by higher (by 32 %) antioxidant activity markers of lyophilized brain tissues, and by free radical oxidation lower intensity (by 13 %) and biomolecules oxidative modification rate (by 16 %) in these lyophilized tissues. Thus, the advisability of using DDW neuroprotective effects in cerebral circulation disturbance in experimental and clinical practice.

Keywords: DDW, hypoxia, brain, catalase, SOD.

INTRODUCTION

One of the modern neuroscience central directions is new ways development and research for brain protection from damage, including those induced by acute hypoxia. Ischemic brain damage is accompanied by a number of glutamate-calcium cascade pathobiochemical reactions, during all stages of which disadaptation at the cellular level and activation of free radical oxidation processes are observed, manifested, for example, by reactive oxygen species hyperproduction [1]. Free radicals and reactive molecules excessive generation, especially against the backdrop of antioxidant defense system inadequate activity, leads subsequently to peroxidation intensification in ischemia/hypoxia and causes damage to biological molecules (lipids, proteins, nucleic acids), integrative intracellular and intracellular signaling processes modification [2, 3], causing an irreversible metabolic changes cascade in brain structures [4], which violates the most of vital processes in organism [5].

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At present, attempts are being made to develop and implement effective neuroprotective agents aimed at reducing damage to brain tissue and restoring central nervous system functional activity in experimental and clinical practice [6-10]. Therefore, in modern fundamental and applied medicine and neurobiology, search and development of protecting neurons new ways under various pathological conditions associated with brain hypoxia are highly relevant [11]. Also, of particular interest for study are agents with antioxidant and cytoprotective activity [12-14], which have potential to correct arising disorders [15-19].

One of the new agents that meets above requirements is water with a modified isotope D/H composition, taking into account the latest scientific literature data on various deuterium concentrations effect on nervous tissue metabolic and functional activity [20-22].

Water with a modified isotope D/H composition with depleted deuterium content has an immunomodulating properties [23-26], which can be very useful while correction hypoxic conditions in which inflammatory

processes are observed [27]. Recent research dataset showed deuterium depleted liquid medium ability to change blood and tissues isotopic D/H composition and thus increase organism's defense systems potential [28-30]. Also, changes in deuterium and protium balance in water can modify mechanisms implementation that mediate long-term memory [31].

In view foregoing, purpose of this study was to investigate prooxidant-antioxidant system in the blood and brain homogenates functional activity in rats with acute hypoxia model with different D/H ratios in drinking diet.

MATERIALS AND METHODS

Animals and Experimental Design

The study was carried out on 24 Wistar rats males 3 months old (weighing in diapason from 253 to 286 g) obtained from Federal State Budgetary Institution Of Science "Scientific Center For Biomedical Technologies Of The Federal Medical And Biological Agency «Andreevka» branch" (Moscow region, Solnechnogorsk district, Andreevka settlement) and quarantined for 10 days, then randomly divided into 3 groups:

- group 1 - intact rats (n = 8), water diet based on mineralized water with a deuterium concentration equal to natural (–11 ‰) for 8 weeks (hypoxia was not modeled);
- group 2 - rats (n = 8), water diet based on mineralized water with a deuterium concentration equal to natural (–11 ‰) for 8 weeks, with acute hypoxia model on the 57th day of experiment;
- group 3 - rats (n = 8), water diet based on mineralized deuterium depleted water (–665 ‰) for 8 weeks, with acute hypoxia model on the 57th day of experiment.

During experiment, all animals were kept under standard vivarium conditions with free access to water and food in plastic TECNIPLAST type IV S cells, in one cage 4 rats were placed (according to animal placement norms) [32]. Keeping animals conditions were standardized: temperature - (20±3) °C, humidity - (48±2) %, day/night mode (from 6.00 to 18.00 / from 18.00 to 6.00). Birch shavings were used as a bedding. During experiment, all animals consumed standard concentrated mixed fodder in accordance with GOST R 50258. Experiment was carried out in accordance to requirements of the Ministry of Health of the Russian Federation «On Approval of Rules for Laboratory Practice» (June 19, 2003), the Rules of Laboratory Practice (GLP), the Helsinki Declaration (2000) and the Directives of the European Community 86 / 609EEC.

Obtaining of Deuterium Depleted Water

Deuterium depleted water was obtained by an electrolytic separation method at facility developed at the Kuban State University (Patent of the Russian Federation No. 2521627, published: 07.10.2014). Water mineralization was carried out by adding salts to achieve a physiologically complete mineral composition that was identical in water with a deuterium content of –665 ‰ and –11 ‰. DDW (deuterium depleted water) mineralization was: hydrocarbonate by 144-180 mg, sulfate - less than 1 mg, chloride by 60-76 mg, sodium by 6 mg, potassium by 3 mg, calcium by 50-58 mg, and magnesium by 50-58 mg. In addition, all rats received standard concentrated mixed fodder (GOST R 50258-92, Russian Federation) *ad libitum*.

NMR Spectroscopy and Isotope Ratio Mass Spectrometry

Deuterium concentration determination in blood plasma was carried out with a JEOL JNM-ECA 400MHz pulsed NMR spectrometer [33]. Measurements calibration in liquids was performed in relation to international standards Vienna Standard Mean Ocean Water (VSMOW [34]: 155,76 ppm or δD=0 ‰) and water corresponding internal standard.

Brain samples were lyophilized in a vacuum freeze dryer “LS-1000” (Prointech, Russian Federation) [35]. Laboratory animals lyophilized brain isotope composition was determined on a DELTA^{plus} mass spectrometer (Finnigan, Germany) [36]. Solid samples were folded in a metallic foil and placed in the combustion chamber using a CARLO ERBA AS 200-LS automatic sampler. Changes in isotope composition were expressed per mille (‰; one one-thousandth) using the standard delta notation δ:

$$\delta = (R_{sample}/R_{stand} - 1) \times 1000\ (‰),$$

where R is the isotope ratio, and R_{sample} and R_{stand} are the D/H ratio in the investigated probe and standard (–11 ‰). The measurements in the solid-state samples were calibrated with respect to an international sample (IAEA-CH-7; δD = –100.3 ‰) and different internal verified standards.

In Vivo Research

Acute hypoxia with hypercapnia was modeled by placing rats in a hermetically sealed 1-liter vessel

where rats were under continuous monitoring until first agonial sigh appearance, next rats were taken out and placed back into cages. One day after hypoxia modeling, rats decapitation was carried out under general anesthesia performed by Zolilet 100 (Virbac, France) in dose 15 mg per kg of rat body weight intramuscularly, then brain was removed and placed for 24 hours in liquid nitrogen. Then sample weighing 50 mg of brain per 1 ml of phosphate buffer (pH 7.4) was prepared. At the same time, while preparing the sample, brain tissue was ground in liquid nitrogen.

Biochemical Analysis

Blood sampling in a volume of 2.5 ml was performed from the cervical artery. The following biochemical parameters were evaluated in the blood plasma: activity of catalase, glutathione peroxidase (GPX), glutathione reductase (GSR), superoxide dismutase (SOD) in the hemolysate, products reacting with thiobarbituric acid (TBV), SH-groups content, in blood plasma - total antioxidant activity (TOA), luminol-dependent H_2O_2-induced chemiluminescence outburst area (CL).

Catalase activity determination method was based on recording hydrogen peroxide (H_2O_2) utilization rate in a reaction mixture into which 200 µl of erythrocytes hemolyzate (1:200 dilution) was added. H_2O_2 utilization rate was judged by severity of decrease in reaction mixture extinction at 260 nm, at which hydrogen peroxide had light absorption maximum [37]. In the course of experiment, hemolysate in quantity 200 µl was added to 0.3 % hydrogen peroxide solution in a volume of 2.5 ml, the reaction was carried out for 10 minutes at temperature 37°C, after which it was stopped by applying 280 µl of trichloroacetic acid 50 % solution. Simultaneously with the experimental, control samples were placed in which trichloroacetic acid was added before hemolysate was added. After centrifugation for 10 minutes at 3000 rpm, the supernatants from control and test samples were photographed at wavelength of 260 nm against trichloroacetic acid 5% solution. Enzyme activity was expressed in millimoles of H_2O_2, destroyed in 1 minute in 1 liter of hemolysate, or mmol / (min·l).

SOD activity was determined using a method based on quercetin autooxidation inhibition as a result of SOD presence in erythrocytes hemolysate. With SOD participation, the superoxide anion radical that is formed during quercetin solution oxidation, initiated by N,N,N_1,N_1-tetramethylethylenediamine introduction in O_2 presence [38], occurs dismutation.

To carry out this enzymatic reaction, 3 ml of 0.015 M sodium potassium phosphate buffer (pH = 7.8), 100 µl of washed red blood cells hemolysate (1:50 dilution), 200 µl of 25 µM quercetin solution in dimethyl sulfoxide were added to the cuvette. To initiate the reaction, 100 µl of N, N, N1, N1-tetramethylethylenediamine 28 mM solution was added. Reaction mixture optical density was first measured after 15 seconds and a second time 15 minutes after reaction start at a wavelength of 406 nm. Enzyme activity was expressed as a percentage of quercetin oxidation inhibition (%), which was calculated by the formula: A = (Econ-Eexp) / Econ · 100, where Econ is the difference between quercetin concentrations after 15 seconds and 15 minutes in control sample, Eexp is the difference between quercetin concentrations after 15 seconds and 15 minutes in test sample, 100 % (%).

Also for more in-depth study of antioxidant protection mechanisms in blood and brain tissues, integral indicator CAT/SOD (CAT activity/SOD activity) was calculated.

GPX activity was determined by method based on the ability of GPX to provide the glutathione reduced form reaction with tert-butyl hydroperoxide. Enzyme activity was assessed by change glutathione reduced form concentration in two samples: one sample was measured before incubation with model substrate, second sample was measured after incubation. Glutathione reduced form concentration was evaluated by a color reaction with 5,5'-dithiobis (2-nitrobenzoic) acid. For this reaction, 0.2 ml of hemolysate (diluted 1: 200) was added to 0.73 ml of buffer. The resulting mixture was incubated at 37°C for 10 minutes. Then reaction was initiated by introducing into reaction system 70 µl of tert-butyl hydroperoxide 0.14 % solution; after incubation for 5 minutes at 37°C temperature, reaction was stopped by applying 0.2 ml of trichloroacetic acid 20 % solution. Tert-butyl hydroperoxide solution in control samples was added only after protein was precipitated with trichloroacetic acid. Then samples were centrifuged at 3000 rpm for 1 minute for 10 minutes. Reduced glutathione concentration was determined in 0.1 ml supernatant by adding it to a 2.65 ml Tris-HCl buffer (pH = 8.5) containing 0.01 % ethylenediaminetetraacetate and 25 µl methanol solution of 5,5'-dithiobis (2-nitrobenzoic acid). Further samples were mixed and photometricated at 412 nm wavelength against distilled water [39]. GPX activity calculation in hemolysate was carried out by taking into account the changes in glutathione reduced form content in these samples

before and after their incubation with tert-butyl hydroperoxide. Enzymatic activity was expressed in micromoles of glutathione concentrations difference in experimental and control samples per minute in 1 liter of hemolysate, or μmol/(min·l).

GSR activity was determined by detecting a decrease in nicotinamide adenine dinucleotide phosphate (NADP·H) concentration. Method was based on oxidized glutathione (GS-SG) NADP·H-dependent catalytic conversion to its reduced form (GSH). Process intensity was estimated by decrease rate in samples optical density, wavelength being 340 nm, since there is NADP·H solution light absorption maximum. For this reaction, 0.05 ml of hemolysate (dilution 1:200) were added to the cuvette with reaction system (containing: 1.8 ml 0.1 M potassium phosphate buffer (pH = 7.0) and 1 mM EDTA solution and 0.1 ml glutathione oxidized form). 3 minutes after the initiation of this reaction by adding 0.1 ml of NADP·H solution, test solution extinction was measured at a wavelength of 340 nm against water. The enzyme activity was expressed in micromoles of NADP·H concentrations difference before and after incubation for 1 minute in 1 liter of hemolysate, or μmol / (min·l) [40].

SH-groups content was determined by method based on thiol groups ability to react with 5,5'-dithio-bis (2-nitrobenzoic acid) to form a colored thionitrophenyl anion having an absorption maximum at 412 nm [41]. SH-groups concentration was determined in 600 μl of erythrocyte hemolysate (1:10 dilution) after deproteinization by 200 μl sulfosalicylic acid 20 % solution.

A quantitative assessment of erythrocyte biomolecules oxidative modification basal and Fe^{2+}-induced products content was carried out using method [42], based on these products interactions, contained in erythrocytes and brain tissues, with thiobarbituric acid to form a colored complex, which color intensity was measured photometrically at a wavelength 450 nm and 532 nm. Erythrocytes oxidative modification Fe^{2+}-induced products measurement in order to assess the their biomolecules stability to the peroxidation process was carried out by introducing in reaction system with membrane cells of Fe^{2+} ions with their final concentration 0.1 mmol/l. Total amount of biomolecules oxidative modification products (measured at 450 nm and 532 nm using a UNICO single-beam scanning spectrophotometer model 2800, Unico Inc., USA) was expressed as a thiobarbituric value (TBV) in optical density unit.

Blood plasma total antioxidant activity determination was performed by amperometric method on antioxidant activity analyzer "Tsvetiauza-AAA-01" (JSC NPO "Khimavtomatika", Russia) [43], based on measurement electric current produced during substrate oxidation (dilution 1: 100 in a 2.2 mM H_3PO_4 solution) at a specified voltage (1.3 V) on carbon disulfide working electrode surface and then comparing resulting signal recorded in nanoamperes per second (nA·s).

Free radical oxidation intensity study in the blood plasma was carried out by luminol-dependent H_2O_2-induced chemiluminescence by determining outbreak area on the LT-01 chemiluminotester (Horos, Joint Venture Soviet-Swedish Company, Russian Federation) using developed specialized hardware and software complex [44].

Prooxidant-antioxidant balance state in rats brain tissue was also studied. On a torsion balance (TV 500, Russia), 300 mg of tissue wiped in a mortar, cooled with liquid nitrogen, was weighed and placed in 6 ml of phosphate buffer 0.067 M (pH = 7.4). It was homogenized by shaking on Vortex (MSC-3000, Latvia) for 10 minutes, centrifuge for 15 minutes at 5000 g and 4°C on a Hermle Z 36 K centrifuge (Germany). Supernatant was obtained and taken into an eppendorf tube, in which further the activity of catalase, GPX, GSR, SOD was determined; the amount of products reacting with thiobarbituric acid (TBV); SH-groups content; total antioxidant activity (AOA), according to methods described above, in which instead of hemolysate, brain tissues supernatant was used in an equivalent manner, as well as the area of the Fe^{2+}-induced CL flare according to the procedure [45]. To study Fe^{2+}-induced chemiluminescence, 1 ml resulting homogenate was added to 18 ml phosphate buffer, heated to 37°C. Next, luminescence (oxidation) was initiated by the addition of 1 ml $FeSO_4$ solution. Luminescence intensity was measured for 10 minutes.

Statistical Analysis

The reliability of the differences in the mean values (M) found between the groups was statistically evaluated using a nonparametric U-test (Mann-Whitney), the difference was considered reliable for $p < 0.05$.

RESULTS

Biochemical Analysis of Blood

Obtained data revealed that in blood during hypoxia development the greatest disturbances are observed in functional activity of antioxidant defense enzymes first

and third lines. Erythrocytes SOD activity increased in group 2 by 53.8 %, and in group 3 by 27.6 % (Table **1**), which indicates to increase in their ability to utilize the superoxide anion radical when consuming deuterium depleted water. Such changes can increase group 3 rats resistance to oxidative damage while hypoxic conditions development. At the same time, in thiol cycle enzymes activity, there was a significant decrease in activity as a GPX in comparison with the control group, more pronounced in group 3 (by 44.3% in comparison to group 2) and activity of GSR by 43.8 % in the group 2 and by 35.6 % in group 3. Such a higher ratio of GSR and GPX activities show red blood cells increased capacity to regenerate oxidized glutathione in group 3 and indirectly indicates a greater adaptive potential of rats organism while hypoxia development after deuterium depleted water drinking. In addition, decrease in GPX activity can also occur in connection with relatively high catalase activity capable of destroying hydrogen peroxide in a timely manner without need to consume glutathione low molecular weight antioxidant cofactor, which further stabilizes the thiol cycle functioning under oxidative stress conditions against reduced activity of GSR red blood cells background. Despite the relatively high catalase activity, unbalance in 1st and 2nd lines antioxidant protection enzymes activity was noted in a significant decrease in CAT/SOD in erythrocytes integral indicator by 34.2 % in group 2 rats and by 22.9 % in group 3 rats (Table **1**), which can also lead to a decrease in superoxide anion radical dismutation rate, and, consequently, to erythrocyte membranes enhanced free radical destruction (hemolysis) or cysteine SH-groups oxidation in hemoglobin molecule, followed by aggregation by it moat and methemoglobin, causing red blood cells destruction in contact with them in the small capillaries.

In general, in spite of the fact that in both experimental groups animals oxidative stress is formed at systemic level, characterized in groups 2 and 3, respectively (in comparison with control group) by an TBV increase in plasma by 90.8 % and 63.6 % with simultaneous reduced glutathione decrease by 11.9 % and 4.3 %, functional possibilities for impaired metabolism restoration were more pronounced in group 3 rats. Confirmation is a reliable decrease in AOA in blood plasma in group 2 rats by 10.2 %, whereas in group 3 rats there was an AOA increase by 7.8 % in comparison with the control group. In turn, H_2O_2-induced CL plasma area was increased in group 2 by 67.0 % and in group 3 by 43.3 %, that can indicate on significant difference in free radical oxidation processes intensity in rats, drinking water with deuterium concentrarion 154 ppm and 52 ppm, characterized by lower free radicals generation rate in group 3 (by 14.2 % in comparison to group 2).

Isotope Concentration in Blood Plasma

All this results can be explained by a significant decrease in deuterium concentration in the plasma of group 3 rats (by 22.1 and 19.5 times) when they consume DDW (Figure **1**), in comparison to animals in groups 1 and 2, respectively.

Biochemical Analysis of Brain

The changes observed at the local level in brain tissue were characterized by a number of prooxidant-

Table 1: Prooxidant-Antioxidant System Markers Changes in Rats Blood after Different Deuterium Concentration Water Diet

Marker	Group 1	Group 2	Group 3
	Blood		
	M±σ	M±σ	M±σ
CATe	39869±5221	40739±7816	39551±7589
SODe	28.6±4.3	44.0±5.3*	36.5±4.2*,#
CATe/SODe	1431±335	941±243*	1102±284*
GPXe	67.3±16.8	21.9±4.6*	12.2±2.5*,#
GSRe	752±79	423±45*	484±51*,#
TBVe	0.11±0.02	0.21±0.04*	0.18±0.03*
SHe	1.17±0.16	1.03±0.14	1.12±0.15
AOAp	678±103	609±87	731±102#
CL-H_2O_2-p	9.7±1.8	16.2±2.3*	13.9±2.0*

Note: * – p<0,05 in comparison to group 1; # – p<0,05 in comparison to group 2; e – erythrocytes, p – plasma.

antioxidant system functioning features, including a significant decrease in catalase activity in rats second group brain homogenates by 26.7 %, in significant changes absence in comparison with the control in the group 3.

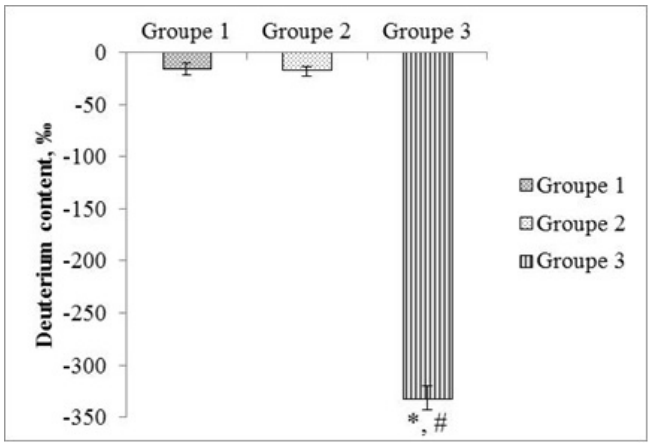

Figure 1: Deuterium comcentration in blood plasma in rats with drinking diet with different deuterium concentration.

Note: * – p<0,05 in comparison to group 1; # – p<0,05 in comparison to group 2.

At the same time, SOD activity was significantly increased in group 2 in comparison to groups 1 and 3 (by 32.9 % and 16.8 %, respectively), that can be characterized as more balanced functional activity enzymes of the first and second line antioxidant protection in neurocytes in rats, drunk deuterium depleted water, than in animals with a usual drinking diet (–665 ‰ in deuterium) while changes development in the nervous tissue caused by hypoxic damages. At the same time, CAT/SOD integral indicator in group 3 was not significantly different from the same value of control group, whereas in group 2, a significant decrease in this indicator was found to be 46.3 % in comparison to group 2 and 39.4 % in comparison to group 3 (Table **2**). It can indicates about increased disruption risk in neutralizing not only free radicals, but also reactive molecules (peroxides), which can forming as a result non-enzymatic decomposition toxic secondary radicals, triggering cascade chain reactions of biological molecules peroxidation in brain tissues.

In contrast to systemic changes at local level, there was also noted increase in the activity of GPX (by 108.5 % and 45.8 %) and GSR (by 12.3 % and 13.8 %), in groups 2 and 3, respectively, in comparison to control group (Table **2**). It can indicate of thiol cycle enzymes significant tension functioning with better GPX and GSR activity ratio in rats, drunk water with a deuterium concentration 52 ppm. In second group rats, a significant imbalance in antioxidant defense enzymes activity was showed by the greatest decrease in reduced glutathione content in comparison with groups 1 and 3 (by 28.1 % and 41.6 %), as well as by smaller AOA brain homogenate level (by 21.9 % and 24.7 %) and by elevated CL index (by 23.8 % and 14.9 %), reflecting the lowest nervous tissue resistance to hypoxia in animals with normal drinking diet, in contrast to rats, drunk deuterium depleted water. All of the above is accompanied by a significant increase in biomolecules oxidative modification products content in brain homogenates in third group rats by 65.2 % and 18.7 %, in comparison to the same markers in groups 1 and 3, respectively (Table **2**), that confirms more significant decay neurocytes cell structures while hypoxia modelling in rats with normal drinking diet.

Table 2: Prooxidant-Antioxidant System Markers Changes in Rats Brain Homogenates after Different Deuterium Concentration Water Diet

Marker	Group 1	Group 2	Group 3
	Brain tissue		
	M±σ	M±σ	M±σ
CATb	15003±2549	10996±1718*	15581±1292#
SODb	41.6±11,0	55.3±11.4	47.3±9.8
CATe/SODb	387±139	208±64*	343±86#
GPXb	59±8	123±16*	86±10*,#
GSRb	65±13	73±11	74±12
TBVb	0.23±0.02	0.38±0.03*	0.32±0.02*,#
SHb	1.21±0.16	0.87±0.09*	1.49±0.17#
AOAb	293±54	229±38*	304±52#
CLb	454±52	562±57*	489±60#

Note: * – p<0,05 in comparison to group 1; # – p<0,05 in comparison to group 2; b – brain tissue.

Isotope Concentration in Brain

At the same time, there was a significant decrease in deuterium content in lyophilized brain tissue in group 3 animals (by 5.3 and 5.9 times), in comparison to groups 1 and 2 (Figure **2**).

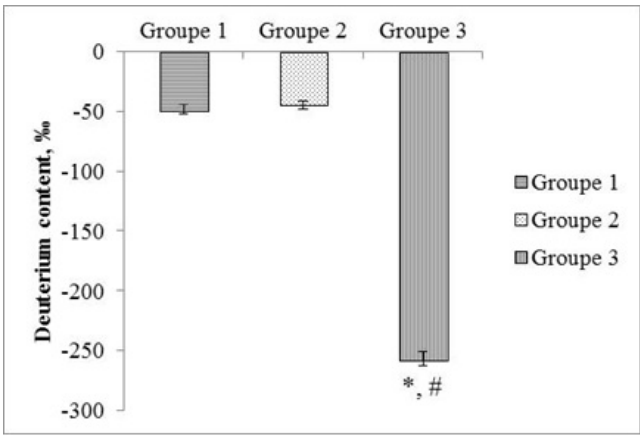

Figure 2: Deuterium concentration in lyophilized brain tissue in rats with drinking diet with different deuterium concentration.

Note: * – p<0,05 in comparison to group 1; # – p<0,05 in comparison to group 2.

DISCUSSION

According to obtained results, it is necessary to emphasize significantly greater sensitivity of nervous tissue cellular components in comparison to erythrocytes to oxidative damage in hypoxia and intensification of free radical processes conditions. Oxidative damage reason is both a more pronounced imbalance in enzyme antioxidant system first and second lines activity at the local level, and, apparently, compensatory increase in GPX functional activity in nerve cells, accompanied by low molecular weight thiol-containing antioxidant factors depletion and decrease their common AOA. At the same time, deuterium depleted drinking diet in rats lead to significant decrease in the most of these pathological biochemical shifts and increase in adaptive potential both on tissue and organism levels [46, 47], increasing nervous tissue resistance and red blood cells to acute hypoxic damage.

Obtained data find some confirmation in recent researches conducted by a number of authors, including a hypothesis about the ability of low deuterium concentrations in water to change DNA sensitivity to oxidizing factors effects in radiation and chemotherapy [48-50], which lead to increased education hydroxyl radical [51, 52]. Other authors studied changes in catalase activity in blood, GSR, GPX and SOD activity in erythrocytes, as well as malonic dialdehyde and glutathione content in rat plasma when they drunk water with a low deuterium content [53], which revealed the prooxidant effect presence of low deuterium concentrations in drinking water at short-term effects and adaptive increase in antioxidant system functional activity during long-term deuterium depleted water consumption. Also it was shown protective effect of low deuterium concentrations in water during intoxication induced by cadmium chloride in Wistar rats, it was noted decrease in cellular structures oxidative damage in conditions of preventive consumption depleted in deuterium drinking ration [54]. In addition, prolonged water with a modified isotope composition (with a deuterium depleted content) consumption in the background of chromium salts intoxication led to not only decrease in biomolecules peroxide modification products, but also to increase in the reduced SH-groups in the blood in comparison to animals, exposed to toxic effects and received a drinking diet with a natural D/H ratio [55]. Some authors explain preventive effects of water consumption with a low deuterium content, accompanied by a lipid peroxidation decrease [56], by exposure to generation active oxygen species rate and nitric oxide production (NO) by changing enzymes activity and expression that regulate free radical exchange in organism [57, 58].

In general, reduction effects in the D/H ratio at the cellular and tissue levels, in the most of studies performed by various scientific groups, are characterized by antioxidant defense mechanisms activity increase, leading to decrease free radical oxidation intensity and biomolecules peroxide modification expression, which allows us to consider a drinking diet with a deuterium depleted water as one of neuroprotection methods in acute hypoxia model in rats. All of the above results obtained in this research work are also the basis for neuroprotective effects of DDW clinical study in patients with impaired cerebral circulation. All of the above results obtained in this research work are also the basis for DDW neuroprotective effects clinical study in patients with impaired cerebral circulation.

CONCLUSION

Based on carried out studies, it should be noted that DDW (52 ppm) consumption by laboratory animals, contributes to antioxidant protection enzymes (catalase, SOD, GPX and GSR) more balanced work in blood, including reducing hydrogen peroxide

uncontrolled formation risk and glutathione oxidative modification, which is characterized by a large (by 20 %) blood antioxidant potential, and is also accompanied by a decrease free radical oxidation intensity in blood plasma and peroxide modification biomolecules rate in erythrocytes.

In rats consuming DDW, there was a lack of disturbances in enzymes 1st and 2nd lines antioxidant protection activity and adaptive changes in thiol cycle enzymes activity, while acute hypoxia development leading to increase (by 71 %) reduced thiol-containing compounds concentration, which reduces damage risk to nerve cells under hypoxic conditions.

In addition, changes in SH-groups in the blood and brain tissues may reflect conformational changes in proteins when administered DDW to rats drinking diet, which may be due to different HDO content in macromolecules (proteins) hydration shell, resulting in different packing densities folding, and, consequently, a change in their native characteristics, which can play a role in cells adaptation under hypoxic effects conditions. Neuroprotective effect presence is also confirmed by higher (by 32 %) brain homogenates antioxidant activity markers, and by free radical oxidation lower intensity (by 13 %) and biomolecules oxidative modification rate (by 16 %) in these homogenates. It shows the advisability of studying the DDW neuroprotective effects in the disturbance of cerebral circulation.

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