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DYNAMICS OF PRO-OXIDANT/ANTIOXIDANT HOMEOSTASIS DURING ACUTE CEREBROVASULAR DISORDERS (EXPERIMENTAL MODELING)

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Abstract

Background. Acute cerebrovascular accidents, particularly ischemic stroke, are a leading cause of mortality and long-term disability worldwide. Oxidative stress and disruption of antioxidant defense systems play a central role in ischemic brain injury, yet the temporal dynamics of pro-/antioxidant imbalance remain insufficiently characterized.

The aim. To assess changes in superoxide dismutase, catalase, citrate, diene conjugates, and total antioxidant activity in brain tissue homogenates and blood serum of rats with experimentally induced ischemic injury, in order to determine temporal patterns of oxidative and antioxidant imbalance.

Materials and methods. The study was conducted on 60 white non-linear rats. Focal cerebral ischemia was induced via endovascular occlusion of the middle cerebral artery according to the Longa model. SOD activity was measured using the nitroblue tetrazolium reduction method, catalase activity by hydrogen peroxide decomposition, DC levels by UV absorption at 232 nm, and TAA via malondialdehyde formation. All experiments were conducted in accordance with GLP and ethical guidelines. Statistical analysis was performed using Student's t-test and Fisher's criterion, with $p<0.05$ considered significant.

Within the first day of ischemic injury, TAA in serum increased by 49% relative to intact controls, indicating an early compensatory activation of antioxidant systems. Brain tissue showed decreased SOD and catalase activity with elevated citrate and DC levels, reflecting enhanced lipid peroxidation. By day 14, serum TAA sharply declined (16.3% below intact controls), while catalase activity remained low and DC levels continued to rise. Persistent SOD hyperactivation alongside inadequate catalase activity suggested accumulation of hydrogen peroxide and progressive oxidative stress.

Conclusions: Acute cerebral ischemia induces a pronounced pro-oxidant shift centrally and systemically, triggering early oxidative damage. Prolonged ischemia depletes antioxidant defenses, exacerbating oxidative stress and tissue injury. The imbalance between SOD and catalase activities promotes hydrogen peroxide accumulation and may serve as a predictive marker of neurodegenerative processes following ischemic stroke.

Keywords: acute cerebrovascular disorder; oxidative stress, antioxidant defense; superoxide dismutase; catalase; diene conjugates; total antioxidant activity

Introduction. Acute cerebrovascular accidents continue to dominate the structure of cerebrovascular disorders. Globally, stroke ranks second among the causes of mortality, accounting for 11.6% of all deaths, with ischemic stroke (IS) being the most prevalent type (62.4% of all cases) [1, 2]. This form of brain injury leads to neuronal death and long-term disability in adults, creating a significant medical, social, and economic burden. According to current projections, one in four individuals over the age of 25 is at risk of experiencing a stroke during their lifetime, with the probability of an IS being 18.3%. The World Health Organization predicts that between 2010 and 2050 the number of stroke cases will more than double [2].

Immune mechanisms, endothelial dysfunction, and oxidative–nitrosative stress play key roles in the development of ischemic damage; however, several triggers of these

processes remain insufficiently understood.

It is well known that in the development and progression of pathologies caused by impaired cerebral circulation, the enhancement of free-radical oxidation of biomolecules plays a particularly significant role, primarily due to the high sensitivity of the brain to the damaging effects of reactive oxygen species (ROS). Under these conditions, activation of the body's antioxidant defense system may serve as an important adaptive mechanism, with superoxide dismutase and catalase occupying central positions. These enzymes neutralize primary ROS that otherwise participate in reactions leading to the formation of secondary, more reactive radicals and toxic reactive molecules [3, 4].

The aim of the study. To assess the changes in superoxide dismutase, catalase, citrate, diene conjugates, and total antioxidant activity in brain tissue homogenates and blood serum of rats with modeled ischemic injury in order to determine the temporal features of oxidative and antioxidant imbalance.

Materials and methods. The study was performed on white 60 non-linear rats. Acute cerebrovascular disorder we investigated in the ischemic stroke model in rats which was reproduced using a model of endovascular occlusion of the middle cerebral artery (focal ischemia) according to E. Z. Longa. The rats were pre-anesthetised, and the surgical field was treated with a 0.05% chlorhexidine solution. After that, an incision was made in the neck area, and the common carotid artery, external carotid artery, and internal carotid artery were isolated on the right side. The common carotid artery was clamped with a vascular clip, and a No. 3 vicryl ligature was applied to the external carotid artery. The internal carotid artery was cut with scissors at a distance of 3-5 mm from the bifurcation. A 0.25 mm diameter nylon thread coated with silicone and treated with heparin solution was inserted through a segment of the internal carotid artery into the external carotid artery to a depth of 19-21 mm and fixed with a vascular clip. Blood flow was blocked for 60 minutes, after which the thread was removed. After that, the internal carotid artery was closed by coagulation until completely sealed, and the vascular clips were then removed. At the end of the operation, the incision was sutured with Vicryl No. 4 and treated with a 5% solution of brilliant green. After the operation, continuous thermometry was performed with the temperature maintained at a physiological level using infrared lamps. During the operation, body temperature was maintained using a heating pad. The average duration of the operation was 7-10 minutes [5, 6].

Superoxide Dismutase (SOD) Activity was determined using the nitroblue tetrazolium (NBT) reduction method in the presence of phenazine methosulfate. The principle of the

method is based on the reduction of NBT by superoxide radicals generated in the reaction with phenazine methosulfate. Absorbance was measured at a wavelength of 540 nm using a spectrophotometer in a cuvette with a 0.5 cm optical path.

Catalase Activity was assessed according to the method of Girin S.V., which is based on the reduction of hydrogen peroxide content in the incubation medium, as catalase decomposes hydrogen peroxide. Catalase activity in the experimental sample was measured relative to the control every 30 s for 3 minutes at room temperature at a wavelength of 230 nm**.** Diene Conjugates (DC) Levels. The primary products of lipid peroxidation are lipid hydroperoxides, which form conjugated dienes in fatty acid molecules. Lipid extracts containing hydroperoxides of polyunsaturated fatty acids with such conjugated diene structures absorb in the UV spectrum at $\lambda = 232$ nm**.** Results were expressed as optical density units per 1 mg of lipids or 1 mL of serum.

Total Antioxidant Activity (TAA) was measured using a method based on spontaneous peroxide oxidation leading to the formation of one of the end products, malondialdehyde. The amount of malondialdehyde formed was used as an indicator of the overall antioxidant activity of blood serum.

All manipulations with animals were carried out in accordance with GLP requirements, the recommendations of the State Expert Centre of the Ministry of Health of Ukraine, the General Ethical Principles of Animal Experiments (Ukraine, 2001), the Law of Ukraine of 21 February 2006 No. 3447-IV, as amended "On the Protection of Animals from Cruel Treatment", the resolution of the First National Congress on Bioethics (Kyiv, 2007), and the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes [7].

Statistical analysis of the obtained results was performed using the Statistica 10.0 software package. The significance of differences between the indicators of untreated animals and experimental groups was assessed using Student's t-test and Fisher's criterion. A p-value<0.05 was considered statistically significant.

Results and discussion. It was established that during the assessment of pro-/antioxidant system markers within days 1 to 14 of experimental ischemic injury, significant alterations in the antioxidant defense system were detected both in the brain tissue homogenate and in blood serum as early as the first day.

The TAA of blood serum increased on average by 49% on day 1 of ischemic injury compared to the intact control group. These findings indicate a pronounced protective response involving the activation of stress-limiting systems in reaction to prooxidant

overload. However, this compensatory reaction is characteristic only at the early stage of the experiment, when the antioxidant system has not yet been depleted (Table 1).

Table 1

Study of the Prooxidant–Antioxidant Balance in the Blood Serum and Brain Tissue Homogenate of Rats with Experimental Cerebral Ischemia (M±m)

Indicator	Intact animals (n=12)	Animals with acute cerebrovascular disorders	
		1st day (n=12)	14th day (n=12)
Brain Tissue Homogenate			
Citrate, mmol/L	0,290±0,013	0,739±0,016*	0,805±0,020*
SOD, units/g tissue	2,7±0,3	1,2±0,8*	1,5±0,6*
Catalase, U/g tissue	32,5±2,1	21,3±3,6*	28,2±4,1*
Diene Conjugates (DC), μmol/L	7,89±0,32	13,41±1,25*	17,89±1,35*/**
Blood Serum			
Citrate, mmol/L	0,435±0,019	1,31±0,035*	1,45±0,04*/**
Total Antioxidant Activity (TAA), nA·s	1445,4±35,25	2155,2±31,8*	1210,5±19,2**
SOD, activity units	0,13±0,06	0,08±0,01	0,09±0,02
Catalase, activity units	1,79±0,01	1,28±0,03*	1,09±0,05**
Diene Conjugates (DC), μmol/L	10,21±0,41	16,34±0,56*	17,67±0,61*

Notes:

1. n – number of experimental animals in each group;
2. *– $p<0.05$ compared to intact animals;
3. ** – $p<0.05$ compared to rats on day 1 of the experiment.

An increase in the TAA index on the first day of the modeled pathology may be associated with the accumulation of hypercatabolic products and tissue degradation components, represented by oligopeptides and low- and medium-molecular-weight compounds which, on the one hand, exhibit antioxidant properties, but on the other – at high concentrations – demonstrate significant toxicity (urea, uric acid, extra-erythrocytic hemoglobin, myoglobin, etc.). With the prolonged course of ischemic injury, the TAA level in blood begins to markedly decrease, which is likely related to the disadaptation of the antioxidant defense system. By day 14, this indicator reached 1210.5 ± 19.2 nA·s, which was

on average 16.3% lower than in intact animals.

In the brain tissue homogenate, citrate levels increased 2.5 times ($p<0.05$) on day 1 of the experiment compared to intact animals, and 2.8 times ($p<0.05$) by day 14. No statistically significant differences were observed between the two experimental time points. SOD activity decreased by 55.5 % on day 1 compared to intact animals. On day 14, SOD levels remained reduced and amounted to 1.5±0.6 units/g tissue.

Similar changes were observed for catalase concentration in the brain tissue homogenate: on day 1, catalase activity decreased 1.5 times ($p<0.05$), and on day 14 – 1.2 times ($p<0.05$) compared to intact animals.

Conversely, the level of diene conjugates (DC) increased 1.7 times ($p<0.05$) on day 1 compared to intact rats, and 2.3 times ($p<0.05$) on day 14 relative to intact animals, which was also 1.3 times ($p<0.05$) higher than the value obtained on day 1 of the experiment.

At the systemic level (blood serum), rats with modeled pathology showed a 3.0 times increase ($p<0.05$) in citrate levels on day 1 relative to intact animals, and 3.3 times ($p<0.05$) by day 14. Serum SOD levels showed a non-significant decrease both on the first day and at the end of ischemic injury development. Catalase activity decreased 1.4 times ($p<0.05$) on day 1 compared to intact animals, and 1.6 times ($p<0.05$) by day 14. DC levels increased 1.6 times ($p<0.05$) on day 1 relative to intact rats and 1.7 times ($p<0.05$) by day 14.

The activation of SOD is an important defensive mechanism against excessive primary radicals, including superoxide anion radicals, in whose dismutation SOD plays a direct role. However, excessive production of reactive oxygen species may occur involving components of the antioxidant defense system, particularly hydrogen peroxide, which can accumulate when SOD activity is elevated and catalase activity is low – precisely the pattern observed in the modeled pathology [8].

Thus, the analysis of pro-/antioxidant system indicators under conditions of acute focal cerebral ischemia revealed several characteristic changes. On day 1, there was an increase in SOD activity and a decrease in catalase activity in blood serum, while in brain homogenate SOD activity decreased. This pattern reflects insufficient activity of intracellular enzymatic systems responsible for free-radical neutralization, accompanied by the accumulation of secondary products (hydrogen peroxide) and pronounced prevalence of plasma prooxidant activity over antioxidant activity [4, 8].

By day 14, a sharp decline in TAA was observed, while at the cellular level SOD activity gradually increased, catalase activity decreased, and DC levels continued to rise. Therefore, the end of the acute period in this ischemic stroke model was characterized by the

emergence of a trend toward partial normalization of intracellular antioxidant enzyme activity. However, the marked reduction in TAA indicates exhaustion of the antioxidant stress-limiting system under oxidative stress.

This pattern of pro-/antioxidant imbalance likely represents a predictor of the development of neurodegenerative processes in brain tissue.

Conclusions

1. Acute cerebral ischemia induces a pronounced pro-oxidant shift both centrally and systemically. During the first day of ischemia, rats demonstrated significant increases in citrate and diene conjugates in brain tissue and serum, accompanied by decreased catalase activity and suppressed SOD activity in the brain. These changes indicate early mitochondrial dysfunction, excessive ROS formation, and activation of lipid peroxidation processes.

2. Prolonged ischemia leads to depletion of antioxidant defenses and worsening oxidative damage. By day 14, a marked decline in total antioxidant activity was observed – significantly below intact levels—together with persistently low catalase activity and elevated diene conjugates. This reflects exhaustion of both enzymatic and non-enzymatic antioxidant systems, deepening oxidative stress and damaging neural tissue.

3. The imbalance between SOD hyperactivation and inadequate catalase activity promotes accumulation of hydrogen peroxide and may drive neurodegeneration. The combination of increased SOD activity (especially intracerebral), insufficient catalase activity, and rising lipid peroxidation products suggests harmful buildup of H_2O_2. This dysfunctional pro-/antioxidant pattern indicates maladaptation of cellular stress-limiting systems and may represent a predictive marker of progressive neurodegenerative changes after acute cerebrovascular injury.

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Conflict of interest

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