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STATE OF ANTIOXIDANT DEFENCE OF THE SPLEEN PARENCHYMA OF RATS UNDER THE INFLUENCE OF A COMPLEX OF CHEMICAL FOOD ADDITIVES

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The study is aimed at analysing the effect of a complex of food additives (sodium glutamate, sodium nitrite, ponceau 4R) on the state of the antioxidant system of the rat spleen. The experiment was carried out on 70 white rats, which were administered a mixture of food additives in doses below the maximum permissible doses for 1–20 weeks. The biphasic dynamics of antioxidant enzyme activity was established: an initial moderate decrease in catalase and superoxide dismutase activity at 4–8 weeks, followed by a multidirectional imbalance at 12–20 weeks (a sharp increase in catalase activity against a significant decrease in superoxide dismutase activity). The detected disbalance in the system of enzymatic antioxidant defence reflects the development of oxidative stress in the spleen tissues under prolonged exposure to food additives, which can lead to impaired immunological function of the organ and be one of the links in the pathogenesis of the systemic toxic effects of the studied substances.

Key words: oxidative stress, spleen, food additives, superoxide dismutase, catalase, sodium glutamate, sodium nitrite, ponceau 4R.

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СТАН АНТИОКСИДАНТНОГО ЗАХИСТУ ПАРЕНХІМИ СЕЛЕЗІНКИ ЩУРІВ ПРИ ВВЕДЕННІ КОМПЛЕКСУ ХІМІЧНИХ ХАРЧОВИХ ДОБАВОК

Дослідження спрямоване на аналіз впливу комплексу харчових добавок (глутамат натрію, нітрит натрію, понсо 4R) на стан антиоксидантної системи селезінки щурів. Експеримент проведено на 70 білих щурах, яким вводили суміш харчових добавок у дозах нижчих за граничнодопустимі протягом 1–20 тижнів. Встановлено біфазну динаміку активності антиоксидантних ферментів: початкове помірне зниження активності каталази й супероксиддисмутази на 4–8 тижні з подальшим різноспрямованим дисбалансом на 12–20 тижні (різке підвищення активності каталази на фоні значного зниження активності супероксиддисмутази). Виявлений дисбаланс у системі ферментативного антиоксидантного захисту відображає розвиток оксидативного стресу в тканинах селезінки при тривалому впливі харчових добавок, що може призводити до порушення імунологічної функції органу та бути однією з ланок патогенезу системної токсичної дії досліджуваних речовин.

Ключові слова: оксидативний стрес, селезінка, харчові добавки, супероксиддисмутаза, каталаза, глутамат натрію, нітрит натрію, понсо 4R.

The work is a fragment of the research project "Pathogenetic mechanisms of post-stress disorders under conditions of exogenous influences and wartime factors and the search for methods of its correction", state registration No. 0124U003313.

The modern world is characterised by a rapid increase in the use of chemical food additives in the food industry. Monosodium glutamate as a flavour enhancer, sodium nitrite as a preservative, and ponceau 4R as a synthetic colourant are among the most common additives used in food production [11]. Despite the defined permissible daily doses of these substances, their complex intake and cumulative effect of long-term consumption remain insufficiently studied, which is of considerable scientific interest and of important practical importance [1, 8, 9].

Of particular concern is the potential ability of these compounds to induce oxidative stress, a pathological condition characterised by an imbalance between prooxidants and the body's antioxidant system. Oxidative stress is an important link in the pathogenesis of many diseases, including cardiovascular, neurodegenerative, autoimmune and oncological pathologies [5, 6, 13].

The spleen, as a central organ of the immune system and an important component of the reticuloendothelial system, is particularly vulnerable to the effects of food xenobiotics due to its involvement in blood filtration, immune defence and metabolism of foreign substances [2, 14, 15]. The study of the dynamics of changes in catalase and SOD activity in spleen tissues during prolonged administration of a complex of dietary supplements will help to determine the stages of oxidative stress development, identify periods of compensatory activation and depletion of antioxidant systems. Of particular value is the possibility of establishing a link between the duration of exposure and the degree of functional impairment, which can serve as a basis for the development of preventive measures and justification of safe terms of consumption of products containing the studied additives.

Therefore, the study of the dynamics of the antioxidant defence of the rat spleen parenchyma after administration of the complex of sodium glutamate, sodium nitrite and ponceau 4R is an important area of

modern experimental medicine, the results of which will be important for understanding the mechanisms of toxic effects of food additives and developing scientifically based recommendations for their safe use.

The purpose of the study was to analyse the dynamics of changes in the markers of antioxidant defence in the spleen parenchyma under the complex administration of sodium glutamate, sodium nitrite and ponceau 4R.

Materials and methods. The study was conducted on 70 sexually mature white rats weighing 180 to 252 g, which were kept in standard conditions of the vivarium of Poltava State Medical University. The rats were divided into control and 6 experimental groups of 10 animals each. Animals of the control group received saline, and animals of the experimental groups received a mixture of chemical food additives (sodium glutamate, sodium nitrite, ponceau 4R) in single doses: 20 mg/kg of sodium glutamate, 5 mg/kg of Ponceau 4R and 0.6 mg/kg of sodium nitrite in 0.5 ml of distilled water orally, for 1, 4, 8, 12, 16 and 20 weeks. These doses were twice lower than the maximum permissible levels for food. At all other times, the rats had free access to water and standard vivarium chow. Experimental animals were withdrawn from the experiment by an overdose of sodium thiopental followed by spleen sampling for biochemical analysis.

The level of catalase activity was assessed by a method based on the determination of coloured products formed by the reaction of hydrogen peroxide with ammonium molybdate (yellow). The amount of hydrogen peroxide that decomposed in the presence of a sample containing catalase was used to determine the activity of catalase.

The superoxide dismutase activity was determined by a method based on the ability of epinephrine to undergo an auto-oxidation reaction in an alkaline medium with the generation of superoxide anion radical, and this reaction proceeds at a certain rate (V1). In the presence of superoxide dismutase, this rate decreases to a certain value (V2), which depends on the activity of superoxide dismutase. Comparison of V1 and V2 velocities allowed us to judge the activity of superoxide dismutase in the spleen homogenate of the studied groups.

Statistical analysis of the results was performed using a personal computer and IBM SPSS Statistics 26.0 software package, which is used for statistical processing of data from biomedical and epidemiological studies. For multiple comparisons between groups, the Kruskal-Wallis test with post hoc analysis according to the Dunn's test was used. The difference was considered statistically significant if $p<0.05$.

The study was conducted in accordance with the ‘Rules for the Use of Laboratory Experimental Animals’ (2006, Appendix 4) and the Helsinki Declaration for the Humane Treatment of Animals, the Law of Ukraine ‘On the Protection of Animals from Cruelty’ (No. 3447-IV of 21.02.2006).) in compliance with the requirements of the Bioethics Commission of Poltava State Medical University (Protocol No. 208 of 22.09.2022), in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Results of the study and their discussion. In our study, it was found that the catalase activity in the group of intact rats was 0.501±0.002 μcat/g, which was considered the baseline level in our study. In the group of rats treated with a complex of food additives for 1 week, catalase activity was 0.487±0.002 μcat/g, which was not statistically significantly different from the baseline ($p=0.272$). After 4 weeks of dietary supplementation, a slight decrease in catalase levels was found compared to the baseline level to 0.453±0.004 μcat/g ($p=0.001$). A similar trend was observed after 8 weeks of exposure, in which the catalase activity was 0.474±0.002 μcat/g and was lower than the baseline level ($p=0.028$). After 12 weeks of administration of the dietary supplement complex, a significant increase in catalase activity was observed in rats compared to the baseline level ($p=0.003$), reaching 4.322±0.236 μcat/g. Similar changes were observed in rats treated with the dietary supplement complex for 16 weeks ($p=0.001$), where this indicator was 5.772±0.353 μcg/g, and in those treated for 20 weeks ($p=0.002$), where this indicator was 5.103±0.902 μcg/g. Dynamic changes in catalase activity in rats with different exposure durations are shown in the table (Fig. 1).

When analysing the dynamics. it was found that after 1 week of administration. no differences from the baseline were observed ($p=0.272$). Further. after 4 weeks. there was a decrease in catalase activity compared to 1 week ($p=0.028$). which persisted after 8 weeks compared to 4 weeks ($p=0.272$). After 12 weeks of dietary supplementation. catalase activity in spleen tissue increased dramatically compared to 8 weeks ($p<0.001$). and then after 16 weeks continued to increase compared to 12 weeks ($p=0.048$). These changes are maintained after 20 weeks of exposure compared to 16 weeks ($p=0.524$).

Thus. we found that the biphasic dynamics of catalase activity in the rat spleen parenchyma under long-term administration of a complex of food additives (monosodium glutamate. sodium nitrite and ponceau 4R) is characterised by an initial slight decrease in enzyme activity after 4–8 weeks of exposure. followed by a sharp increase after 12–16 weeks and the maintenance of high activity in the longer term.

The activity of SOD in the control group of rats was 10.421±0.761 units. which was used as a baseline. In the group of rats treated with a complex of food additives for 1 week. the activity of SOD was 8.555±0.707 units. which was not statistically significantly different from the baseline (p=0.260). After 4 weeks of dietary supplementation. a slight decrease in the level of SOD was found compared to the baseline level to 6.793±0.450 units (p=0.002). A similar trend was observed after 8 weeks of exposure. in which the SOD activity was 7.502±0.506 units and was lower than the baseline level (p=0.027). After 12 weeks of administration of the complex of food additives. a sharp decrease in SOD activity was observed in rats compared to the baseline level (p<0.001). reaching 0.211±0.004 units. Similar changes were observed in rats treated with the complex of food additives for 16 weeks (p<0.001). where this indicator was 0.297±0.0022 units. as well as in those treated for 20 weeks (p<0.001). where this indicator was 0.248±0.004 units. Dynamic changes in SOD activity in rats with different exposure durations are shown in the table (Fig. 2.)

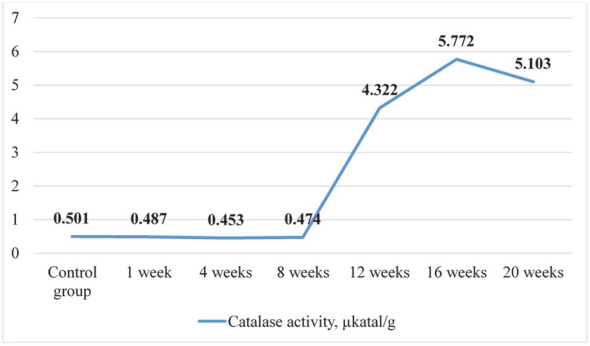

Fig. 1. Catalase activity in the spleen parenchyma of rats with different duration of administration of a complex of food additives.

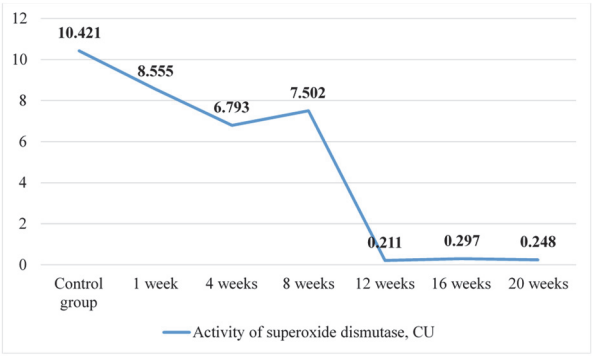

Fig. 2. Activity of superoxide dismutase in rat spleen parenchyma at different duration of administration of food additives complex.

When analysing the dynamics. it was found that after 1 week of administration. no differences from the baseline were observed (p=0.260). Further. after 4 weeks. there was a decrease in SOD activity compared to 1 week (p=0.045). which persisted after 8 weeks compared to 4 weeks (p=0.359). After 12 weeks of dietary supplementation. the activity of SOD in spleen tissue decreases sharply compared to 8 weeks (p<0.001). and then after 16 weeks continues to decrease compared to 12 weeks (p=0.028). These changes persisted after 20 weeks of exposure compared to 16 weeks (p=0.272).

Thus. we observed a gradual decrease in SOD activity at 4–8 weeks of exposure with a sharp drop in enzyme activity during 12–16 weeks and further maintenance of its reduced level of activity.

The analysis of the results obtained on the dynamics of the activity of key antioxidant enzymes in the rat spleen parenchyma under conditions of prolonged administration of a complex of food additives (sodium glutamate. sodium nitrite and ponceau 4R) reveals important patterns of oxidative stress development and the formation of adaptive mechanisms. Particular attention should be paid to the multidirectional nature of changes in the activity of the studied enzymes. which may reflect complex processes of redox homeostasis disruption and compensation mechanisms.

At the early stages of exposure (1–4 weeks). different trends in the activity of the studied enzymes are observed. The activity of catalase after 1 week of administration of the dietary supplement complex remains at a level close to the control values. followed by a slight decrease after 4 weeks. At the same time. the activity of SOD demonstrates a more pronounced reaction to the effects of food additives. with a statistically significant decrease in activity after 4 weeks.

This early reaction of SOD can be explained by its primary role in neutralising superoxide anion radical. which is the primary reactive oxygen species (ROS). SOD is characterised by a higher sensitivity to the inhibitory effect of sodium glutamate and sodium nitrite compared to catalase. which is due to the peculiarities of the structure of the enzyme's active centre and its interaction with toxicants [4]. In addition. in the early stages of exposure to food additives. there is a more intense generation of superoxide anion radical compared to hydrogen peroxide. which leads to the predominant use of SOD molecules and a decrease in its activity [3].

In the middle phase of exposure (4–8 weeks). stabilisation of the reduced activity of both enzymes is observed. Catalase activity continues to decrease slightly. while SOD activity remains at a reduced level. This phase reflects a state of moderate oxidative stress with partial compensation by other antioxidant systems. Under conditions of a moderate decrease in the activity of SOD and catalase in the spleen tissues. the glutathione antioxidant defence system is activated. as well as the synthesis of low molecular weight antioxidants such as ascorbic acid and tocopherol is increased [7].

In the late phase of exposure (8–20 weeks). the most pronounced imbalance in the antioxidant defence system is observed. which is manifested by multidirectional changes in the activity of the studied enzymes. After 12 weeks of exposure. there is a sharp decrease in the activity of SOD. followed by a further decrease and stabilisation at a minimum level. In contrast. catalase activity shows the opposite trend – a sharp increase after 12–16 weeks of exposure and then maintaining a high level.

This imbalance in the enzymatic antioxidant defence system can be explained by several mechanisms. Firstly. prolonged exposure to a complex of food additives has been shown to lead to irreversible modification of SOD molecules through nitrosylation and glycosylation. which significantly reduces its catalytic activity [12]. At the same time. catalase molecules are characterised by higher resistance to such post-translational modifications due to their structure [10]. Secondly. as noted. the activation of the transcription factor Nrf2 under prolonged oxidative stress can be selective for various antioxidant genes.

The detected imbalance in the SOD catalase system is of great functional importance for the development of oxidative stress in spleen tissues. Under conditions of a significant decrease in SOD activity against the background of increased catalase activity. the consistency of the functioning of antioxidant enzymes of the first line of defence is disturbed. An imbalance in the SOD-catalase system in spleen tissues leads to a violation of the functional activity of immunocompetent cells. especially macrophages and T-lymphocytes. which may be manifested by a decrease in their ability to proliferate and produce cytokines. It has been shown that an imbalance in the spleen's antioxidant defence system disrupts the processes of erythrophagocytosis and the disposal of damaged red blood cells. which can lead to anaemia and an increased risk of autoimmune reactions. At the same time. a prolonged imbalance of SOD and catalase activity in the spleen parenchyma contributes to the development of fibrotic changes and impaired microcirculation in the organ [3].

Conclusion

A comprehensive analysis of the dynamics of catalase and SOD activity in the rat spleen parenchyma under conditions of prolonged administration of a complex of food additives revealed the formation of a progressive imbalance in the system of enzymatic antioxidant defence. This imbalance is characterised by multidirectional changes in the activity of the studied enzymes in the late period of exposure: a sharp decrease in the activity of SOD against a significant increase in the activity of catalase. Such changes reflect the complex mechanisms of oxidative stress development and the formation of adaptive responses in response to prolonged exposure to a complex of food additives. The revealed imbalance is of great functional importance for the development of pathological changes in the spleen and impairment of its immunological function. which may be one of the links in the pathogenesis of systemic toxic effects of food additives.

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COLLAGEN FIBERS AND PROTEOGLYCANS IN THE GLIAL SCAR IN THE BRAIN AFTER HEMORRHAGIC STROKE AND UNDER CONDITIONS OF MODULATION OF COMPENSATORY-REPAIR PROCESSES

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In the case of a stroke, a glial scar forms around the affected area. According to the hypothesis, scar formation occurs not only by glial cells but also mesenchymal cells, which should be reflected in the cellular composition and extracellular matrix. The aim of this study was to investigate changes in collagen and proteoglycans content in the glial scar in the brain after hemorrhagic stroke and under conditions of modulation of compensatory-repair processes. A local hemorrhagic stroke was modeled in rats, and histochemical methods were used to study changes in collagen and proteoglycans content in the scar formation areas. An increase in collagen accumulation was observed on days 3, 10, and 30 after the stroke, with a tendency to decrease by day 60. The elimination of the hemorrhage was characterized by the accumulation of macrophages with PAS-positive cytoplasm and an increase in proteoglycans content around the hemorrhage. This indicates the involvement of mesenchymal-derived cellular elements in scar formation. In conditions of granulocyte-colony stimulating factor application, collagen accumulation was significantly lower, as it was with dexamethasone treatment, while isolated dexamethasone action led to scar formation characterized by an increase in collagen and macrophage content.

Key words: hemorrhagic stroke, glial scar, collagen, proteoglycans, dexamethasone, granulocyte colony-stimulating factor.

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КОЛАГЕНОВІ ВОЛОКНА ТА ПРОТЕОГЛІКАНИ У СКЛАДІ ГЛІАЛЬНОГО РУБЦЯ У МОЗКУ ПІСЛЯ ГЕМОРАГІЧНОГО ІНСУЛЬТУ ТА ЗА УМОВ МОДУЛЯЦІЇ КОМПЕНСАТОРНО-ВІДНОВЛЮВАЛЬНИХ ПРОЦЕСІВ

За інсульту формується гліальний рубець навколо ділянки ураження. Його формування відбувається не тільки за участі глії, але і за рахунок клітин мезенхімального походження. Це знаходить своє відображення у клітинному складі та стані позаклітинного матриксу. Метою роботи було дослідити зміни кількості колагенових волокон та протеогліканів у гліальному рубці у мозку після геморагічного інсульту та за умов модуляції компенсаторно-відновлювальних процесів. Щурам моделювали локальний геморагічний інсульт і гістохімічними методами досліджували зміни кількості колагену та протеогліканів у ділянках формування рубця. Виявлено збільшення накопичення колагенових волокон через 3, 10 і 30 діб після інсульту і тенденція до його зниження через 60 діб. Елімінація крововиливу характеризувалася накопиченням макрофагів з ШИК-позитивною цитоплазмою і збільшенням вмісту протеогліканів навколо крововиливу. Це могло свідчить про певну участь клітинних елементів мезенхімного походження в утворенні рубця. За умов введення гранулоцитарного колоніестимулюючого фактору накопичення колагену була достовірно меншим, як і у поєднані його дії з дексаметазоном, тоді як дексаметазон сприяв збільшенням вмісту колагену та макрофагів, що фагоцитували протеоглікани.

Ключові слова: геморагічний інсульт, гліальний рубець, колаген, протеоглікани, дексаметазон, гранулоцитарний колоніестимулюючий фактор.

This study is a part of the research project "Studies of regenerative processes in the brain and nerve trunk under conditions of modulation of accumulation and differentiation of mesenchymal stem cells", state registration No. 0120U101376.

The glial scar formed after a hemorrhagic stroke is a known phenomenon. However, cellular reactions and their dynamics in this process have numerous unexplained issues. In most studies of the glial scar, attention is focused on the reaction of astrocytes, as the predominant cell elements in it, and, to a lesser extent, on microglia, as mediators of the inflammatory reaction. But scar formation is not limited to