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CHANGES IN THE PROOXIDANT-ANTIOXIDANT SYSTEM OF RAT HEARTS UNDER THE COMPLEX INFLUENCE OF FOOD ADDITIVES AND THEIR CORRECTION WITH MELATONIN

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This study presents the results of research on changes in the prooxidant-antioxidant system of rat hearts under the combined effect of food additives and the correction of pathological shifts with melatonin. It was established that the complex influence of sodium glutamate, the synthetic dye Ponceau 4R, and sodium nitrite leads to an increase in lipid peroxidation products in heart homogenates and multidirectional changes in the antioxidant defence system. Initially, these changes manifest as an increase in enzyme activity, followed by a decrease, indicating the development of oxidative stress and the depletion of the enzymatic component of the antioxidant system, with damage mechanisms prevailing over protective mechanisms. The administration of melatonin during the last ten days of the study contributed to a reduction in lipid peroxidation processes and improved the balance of the prooxidant-antioxidant system. Thus, exogenous melatonin is a potentially useful agent for correcting oxidative stress in the myocardium.

Key words: food additives, rats, heart, oxidative stress.

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ЗМІНИ ПРООКСИДАНТНО-АНТИОКСИДАНТНОЇ СИСТЕМИ СЕРЦЯ ЩУРІВ ЗА УМОВ КОМПЛЕКСНОГО ВПЛИВУ ХАРЧОВИХ ДОБАВОК ТА ЇХ КОРЕКЦІЇ МЕЛАТОНІНОМ

У роботі представлені результати дослідження змін прооксидантно-антиоксидантної системи серця щурів при комплексній дії харчових добавок та корекції патологічних зрушень мелатоніном. Встановлено, що комплексний вплив глутамату натрію, синтетичного барвника понсо 4R та нітриту натрію призводить до підвищення у гомогенаті серця рівнів продуктів ліпопероксидації та різноспрямованих змін системи антиоксидантного захисту, що спочатку проявляються зростанням активності ферментів, а потім їхнім зниженням, що свідчить про розвиток оксидативного стресу і вказує на виснаження ферментативної ланки антиоксидантної системи та переважання механізмів пошкодження над механізмами захисту. Введення мелатоніну протягом останніх десяти діб дослідження сприяло зменшенню інтенсивності процесів перикисного окиснення ліпідів та покращенню балансу ланок прооксидантно-антиоксидантної системи. Таким чином, екзогенний мелатонін є потенційно корисним засобом для корекції окислювального стресу у міокарді.

Ключові слова: харчові добавки, щури, серце, окислювальний стрес.

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In recent decades, the chemical and food industries have been rapidly developing, accompanied by the use of a wide range of food additives that are intentionally added to pharmaceutical products, raw materials, semi-finished products, or final goods to extend shelf life, enhance, regulate, and preserve organoleptic properties [5]. The most commonly used food additives, not only in Ukraine but worldwide, that exert toxic effects on human and animal health include sodium glutamate (E621), the synthetic dye Ponceau 4R (E124), and sodium nitrite (E250) [1, 2, 10, 12]. Regular consumption of food products containing a combination of these additives leads to their increased intake into the body, exceeding the maximum permissible consumption levels. This is accompanied by metabolic changes, particularly oxidative stress, which underlies the pathogenesis of many diseases [2, 11, 12, 14]. A nonspecific response of the body to oxidative stress is the activation of lipid peroxidation processes, leading to the accumulation of toxic peroxidation products, destabilization of antioxidant defence, and, consequently, an increase in endogenous intoxication, metabolic disorders, and pronounced morphological and functional changes.

An analysis of recent scientific publications indicates that the effects of each food additive have been studied individually. However, experimental studies on the combined impact of these food additives are scarce and primarily describe the features of morphological remodelling of digestive system organs [1, 10, 13]. Regarding publications dedicated to investigating metabolic disorders underlying morphological changes in the heart under the combined influence of these food additives, they are

practically non-existent. Similarly, there is limited data on the pharmacological correction of pathological changes induced by food additives. It is known that antioxidant-based drugs are used to correct disorders caused by oxidative stress [3]. One of the antioxidants capable of interacting with various free radical oxygen species, enhancing the enzymatic component of the antioxidant system, and exhibiting cardioprotective properties is the pineal gland hormone – melatonin [6, 9, 15]. However, research on pathological changes in the prooxidant-antioxidant system of the heart under the complex influence of food additives and potential correction by exogenous melatonin is limited, which prompted us to conduct our own study.

The purpose of the study was to determine changes in the prooxidant-antioxidant system of rat hearts under the influence of a combination of food additives (sodium glutamate (E621), Ponceau 4R (E124), and sodium nitrite (E250)) and the correction of pathological shifts with melatonin.

Materials and methods. The experiment involved 48 sexually mature male Wistar rats with an average body weight of 210.0±0.67 g, which were divided into three groups. The animals were kept under standard vivarium conditions with a natural light cycle and had free access to food and drinking water. The experiments were conducted during the spring season. The control group (n=6) remained under standard vivarium conditions without additional exposure to any factors. Biological material from the control group was collected at the end of the experiment. Rats in the second group were administered a combination of food additives orally once daily before the main feeding: 0.6 mg/kg sodium nitrite (E250, Uralchem, China), 20 mg/kg sodium glutamate (E621, Multichem, China), and 5 mg/kg Ponceau 4R (E124, Multichem, China), dissolved in 0.5 ml of distilled water. The dosage of the food additives used was half the permissible limit allowed in the food industry by the State Standard of Ukraine and, according to the literature, does not have a negative impact on health [10]. Rats in the third group received the specified combination of food additives, and to correct their effects, melatonin (Vita-Melatonin, JSC “Kyiv Vitamin Plant”, Ukraine) was additionally administered intraperitoneally at a dose of 1.0 mg/kg body weight in 1.0 ml of solvent once daily at 19:00 for 10 days (from days 47 to 56 of the experiment) [9].

For comparative analysis of biochemical parameters, rats were withdrawn from the experiment at different time points: on days 7, 14, 21, 28, 35, 42, and 56 from the start of the study. At each stage, six rats from each group were selected, which is the minimum generally accepted number of animals required for statistical research. Decapitation was performed under thiopental anesthesia (25 mg/kg, intraperitoneally). For further biochemical analysis, the heart was excised from the animals. A 10 % homogenate was prepared from the blood-free heart using an isotonic solution. Metabolic processes in the myocardium were analyzed based on the concentration of free radical lipid peroxidation products such as diene conjugates and malondialdehyde, which are the most informative indicators of oxidative stress, as well as the activity of antioxidant enzymes (superoxide dismutase, catalase) in the heart homogenate [8].

All stages of the study on laboratory animals were conducted in compliance with the principles of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg, 1985) and other international agreements, as well as current national legislation on biomedical research.

Statistical analysis of the obtained results was carried out using methods of variation statistics. The Shapiro-Wilk test was used to check for normal distribution. When the data followed a normal distribution, Student's t-test was applied for comparison of variation series; otherwise, the nonparametric Wilcoxon (Mann-Whitney) U-test was used. Differences were considered statistically significant at $p<0.05$. Statistical calculations were performed on a personal computer using the standard software package “STATISTICA 6” for Windows.

Results of the study and their discussion. The biochemical study results indicate that animals exposed to a combination of food additives exhibit characteristic signs of oxidative stress, manifested by the progressive accumulation of lipid peroxidation products in the myocardium throughout the study. A significant increase in diene conjugates compared to control values was observed on days 7 and 14 of the experiment. Specifically, on day 7, the concentration of diene conjugates in heart tissue homogenates increased by 25.77 % ($p<0.05$), and by day 14, it rose by 35.01 % ($p<0.05$) relative to the control, indicating the stimulation of free radical oxidation processes (Table 1). On day 21, a decrease of 10.58 % in diene conjugates was recorded compared to day 14, which may be interpreted as an adaptive-compensatory response aimed at maintaining functional homeostasis. From day 28 onward, a gradual but slower increase in diene conjugates was observed compared to the first two weeks of the study. By

day 28, the diene conjugate level increased by 26.61 % ($p<0.05$) compared to the control, on day 35 − by 36.69 % ($p<0.05$), on day 42 − by 37.82 % ($p<0.05$), on day 49 − by 39.78 % ($p<0.05$), and on day 56 the concentration reached its peak at 5.02±0.34 mmol/kg, exceeding the control group level by 40.62 % ($p<0.05$).

Table 1

Indicators of prooxidant-antioxidant status in rat myocardium under 56-day exposure to a complex of food additives (M±m)

Animal groups	Indicators studied			
	Diene Conjugates, mmol/kg, n=6	MDA, mmol/kg, n=6	SOD, arbitrary units/g, n=6	Catalase, mkat/kg, n=6
Indicators of Prooxidant-Antioxidant Status in the Control Group Animals				
Control	3.57±0.34	46.31±2.17	65.35±1.92	3.02±0.03
Indicators of prooxidant-antioxidant status under the influence of a complex of food additives				
7th day	4.49 ±0.23*	53.84±2.38*	72.48±1.76*	3.36±0.04*
14th day	4.82±0.31*	57.23±3.16*	77.93±1.84*	3.82±0.04*
21st day	4.31±0.29	56.72±2.78*	73.21±1.32*	3.61±0.06*
28th day	4.52±0.27*	60.15±2.23*	68.19±1.87	3.24±0.05
35th day	4.88±0.22*	62.21±3.02*	63.12±1.68	2.70±0.04*
42nd day	4.92±0.32*	63.07±2.84*	60.39±1.72	2.47±0.05*
49th day	4.99±0.25*	64.12±3.49*	56.05±1.93*	2.32±0.05*
56th day	5.02±0.35*	65.86±3.62*	51.94±1.75*	2.27±0.04*
Food Additives+Melatonin (10th Day)	4.03±0.42	47.92±3.09	70.16±1.81	3.19±0.05

Note: *Significantly different from control ($p<0.05$)*

When determining the content of malondialdehyde, a gradual increase in its concentration was observed. In the group of rats subjected to the experimental conditions, the content of malondialdehyde on the 7th day of the study increased by 16.26 % ($p<0.05$), and by 23.58 % ($p<0.05$) on the 14th day compared to the control values. However, on the 21st day of the study, the level of malondialdehyde slightly decreased but significantly exceeded the control values by 22.48 % ($p<0.05$). From the 28th day until the end of the experiment, the malondialdehyde level continued to rise by 29.89 % ($p\leq0.05$), 34.33 % ($p\leq0.05$), 36.19 % ($p\leq0.05$), 38.46 % ($p\leq0.05$), and 42.22 % ($p\leq0.05$) on the 28th, 35th, 42nd, 49th, and 56th days compared to the intact group values. Thus, the obtained results indicated that even at the end of the study period, the indicators of the prooxidant system status remained high and showed no tendency to decrease, indicating excessive formation and accumulation of lipid peroxidation products and the development of oxidative stress, especially in the later stages of the experiment.

The most important link in protection from free radical processes is the antioxidant system, which consists of antioxidant enzymes and represents a set of protective mechanisms aimed at maintaining and preserving the organism's homeostasis through the regulation of free radical processes. The main enzymes of the antioxidant system that participate in neutralizing free radicals and active oxygen forms, as well as slowing down their formation, are catalase and superoxidedismutase.

According to the results of our studies, the administration of a complex of food additives to the animals caused multidirectional changes in the activity of antioxidant enzymes in the myocardium of rats throughout all periods of the experiment. The dynamics of changes in superoxide dismutase activity were characterized by a gradual increase in its levels by 10.91 % ($p<0.05$) on the 7th day and 19.25 % ($p<0.05$) on the 14th day compared to the control. Therefore, the increase in superoxidedismutase activity during the first two weeks of the study, in our opinion, is a protective reaction of the organism to the intake of food additives, indicating the active involvement of the enzyme in the process of neutralizing free radicals. Starting from the 21st day, we observed a gradual decrease in the activity of the enzyme compared to the intact group. Thus, on the 21st and 28th days, the superoxide dismutase level decreased but still exceeded the control group by 12.03 % ($p<0.05$) and 4.35 %, respectively. On the 35th day, the enzyme level decreased by 3.41 % relative to the intact group, by 7.59 % on the 42nd day, and by 14.23 % ($p<0.05$) on the 49th day. The maximum decrease in superoxidedismutase activity of 20.52 % ($p<0.05$) was recorded on the 56th day of the experiment, indicating suppression of the enzyme and accumulation of lipid hydroperoxides.

The dynamics of catalase activity changes after the administration of the food additives showed a similar pattern. On the 7th and 14th days, an increase in catalase activity was observed by 11.26 % ($p<0.05$) and 26.49 % ($p<0.05$), respectively, which may indicate a compensatory increase in the activity of the enzyme due to the intensification of lipid peroxidation processes and the accumulation of lipid peroxidation products. In later observation periods, the trend reversed. Starting from the 21st day, a decrease in catalase activity was observed in the heart tissue. The results of our biochemical studies showed that on the 21st day of the experiment, the concentration of catalase decreased, but still exceeded the intact group by 19.54 % ($p<0.05$). On the 28th day, further reduction in catalase activity was observed. On the 35th day of the experiment, the catalase level decreased by 10.6 % ($p<0.05$), and on the 42nd day, it decreased by 18.21 % ($p<0.05$) compared to the control. Starting from the 49th day, the reduction in catalase activity was slower and reached its lowest values on the 56th day. Thus, on the 49th day, the catalase level relative to the control decreased by 23.18 % ($p<0.05$), and on the 56th day, it decreased by 24.83 % ($p<0.05$), which provides grounds to claim that the antioxidant defence system was suppressed.

Biochemical analysis of the heart homogenate in the experimental animal group showed that daily administration of melatonin for 10 days contributed to a reduction in the intensity of lipid peroxidation processes. This was confirmed by a decrease in the levels of dienes conjugates and malondialdehyde compared to the group of animals not exposed to the melatonin corrective influence. It was found that the level of dienes conjugates decreased, and by the 10th day of correction, it exceeded the control value by 12.89 %, approaching the control levels. The melatonin correction also contributed to reduction in malondialdehyde concentration. On the 10th day of correction, the malondialdehyde level was nearly at physiological values, fluctuating within 47.92±3.09 mmol/kg, while the intact group had a value of 46.31±2.17 mmol/kg.

Along with the reduction of lipid peroxidation products, we observed a gradual increase in the enzymatic activity of the antioxidant system. Melatonin administration promoted an increase in superoxidedismutase activity. On the 56th day, the activity of the enzyme in the heart homogenate of rats under melatonin correction increased by 35.08 % ($p<0.05$) compared to the group of animals not exposed to melatonin, and it did not significantly differ from the control group values. Melatonin administration also promoted an increase in catalase activity. On the 10th day of correction, catalase concentration in the heart homogenate reached 3.19±0.05 mkat/kg, which exceeded the value of the group (56th day) not subjected to any corrective influence by 40.53 % ($p<0.05$).

Thus, the results of our studies indicate a disruption in the balance between lipid peroxidation processes and the antioxidant defence system. The inability of the animals' endogenous antioxidant system to neutralize lipid peroxidation products leads to changes in the structural and functional properties of cardiomyocyte membranes, which is an unfavourable condition for the functioning of the cardiovascular system [7, 9]. The cardioprotective efficacy of exogenous melatonin was manifested both by a reduction in dienes conjugates and an increase in the activity of the enzymatic link of the antioxidant system. The normalizing effect of melatonin on the antioxidant system of other organs under adverse factors is also reported by other researchers [4, 8, 9].

Conclusion

Summarizing the obtained results of the study, it can be stated that all periods of exposure to the complex of food additives were characterized by disturbances in the functional state of the prooxidant and antioxidant systems, manifested by a statistically significant increase in the levels of lipid peroxidation products (conjugated dienes and malondialdehyde) in the heart homogenate, as well as various changes in the antioxidant defence system. Initially, an increase in the activity of enzymes (superoxidedismutase and catalase) occurred, as a possible compensatory mechanism, but later, their decrease was observed, indicating a disruption of cellular homeostasis and the development of oxidative stress, especially at the later stages of the study. This suggests the exhaustion of the enzymatic component of the antioxidant system and the predominance of damage mechanisms over defence mechanisms. The introduction of melatonin during the last 10 days of the study provided cardioprotection by reducing the activation of lipid peroxidation processes and improving the balance between the prooxidant and antioxidant system components. Thus, exogenous melatonin is a potentially useful tool for correcting oxidative stress in the myocardium.

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