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REACTIVE CHANGES IN THE VESSELS OF THE HEMOMICROCIRCULATORY BED IN THE RETINA OF RATS UNDER THE INFLUENCE OF A COMPLEX OF FOOD ADDITIVES

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The paper examines the effect of a complex of food additives on the vessels of the hemomicrocirculatory bed of the rat retina. Ponceau 4R can induce oxidative stress and inflammation in the retina, potentially leading to damage to retinal pigment epithelial cells. This can potentially cause vision loss and other eye problems. The effect of the complex of nutritional supplements on the resistive link of the hemomicrocirculatory bed is manifested by spasm due to activation of NMDA receptors at week 1, partial compensation due to angiogenesis at week 4, and remodeling of fibrous tissue and loss of elasticity at week 12–16. Regarding the capacitive link, spasm is determined in the early stages, while dilation occurs in the later stages. In the exchange link, ischemic spasm was detected in the early stages of the experiment, endothelial desquamation occurred in the eighth week, and pericyte loss and microaneurysm formation were observed in the 12th to 16th week.

Key words: retina, rats, monosodium glutamate, sodium nitrite, Ponceau 4R, oxidative stress.

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РЕАКТИВНІ ЗМІНИ СУДИН ГЕМОМІКРОЦИРКУЛЯТОРНОГО РУСЛА СІТКІВКИ ЩУРІВ ПІД ВПЛИВОМ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК

У статті розглянуто вплив комплексу харчових добавок на судини гемомікроциркуляторногорусла сітківки ока щурів Понсо 4R може викликати окислювальний стрес і запалення в сітківці, що може призвести до пошкодження клітин пігментного епітелію сітківки. Це потенційно може спричинити погіршення зору та інші проблеми з очима. Вплив комплексу харчових добавок на резистивну ланку гемомікроциркуляторного русла проявляється на 1 тиждень спазмом через активацію NMDA рецепторів, на 4 тиждень – частковою компенсацією за рахунок ангіогенезу, на 12-16 тиждень ремоделюванням фіброзної тканини та втратою еластичності З боку ємнісної ланки визначається спазм на ранніх термінах і дилятація на пізніх. В обмінній ланці на ранніх термінах експерименту виявлявся ішемічний спазм, на восьмому тижні – десквамація ендотелію, на 12-16 тижні втрата перицитів та формування мікроаневризм.

Ключові слова: сітківка, щури, глутамат натрію, нітрит натрію, Понсо 4R, оксидативний стрес.

The study is a fragment of the research project "Restructuring of the organs of the immune, respiratory, and excretory systems under the effect of various exogenous factors (monosodium glutamate, sodium nitrite, ethanol, methacrylate)", state registration No. 0121U108234.

Recent scientific publications have examined the effects of various food additives on organs and physiological systems. However, current data remain insufficient, and studies investigating their combined effects remain scarce [1–3].

The most well-known and widely used flavor enhancer is E-621 – monosodium glutamate (MSG), which intensifies taste perception but also exhibits pathophysiological and toxicological effects. Numerous studies by international researchers highlight its harmful impact [5, 7, 13].

As a result, there is currently no consensus on a safe dosage of this commonly used food additive. Furthermore, the mechanisms underlying the pathogenic and damaging effects of monosodium glutamate remain insufficiently studied.

The general opinion is that food additives can provoke a range of adverse reactions, such as allergic responses, asthma attacks and gastrointestinal disorders. Media reports frequently highlight relationship between certain food additives and the development of cancerous tumors, allergies and other negative outcomes. However, it is important to recognize that the effect of any chemical substance on the human body depends on several factors, including individual characteristics, the quantity of the substance and the duration of exposure [4].

The findings of the studies have shown that most food additives can be classified as completely safe. The safety of food additives has been established through extensive comparative research, and their use is permitted only after thorough testing and approval by the relevant authorities. Over time, as new toxicological or other data emerge and analytical methods develop, government regulations may be updated.

If the daily consumption of E 250 from food does not exceed 0.2 mg/kg, it is considered safe. Sodium nitrite is used as a bronchodilator, spasmolytic and an antidote for cyanide poisoning. It is known that sodium nitrite causes vasodilation, leading to the relaxation of the smooth muscle in arterial blood vessels [10].

Another study published in 2022 focused on the impact of food additives, including E124, on visual processing and attention in children. The research showed that food additives, including colorants, may have a negative effect on visual attention and perception, which could indirectly influence the function of the retina [11].

Noteworthy, the concentrations of E124 used in these studies were higher than those typically found in food products. Nevertheless, these studies raise concerns about the potential negative impact of food colorants on retinal health.

The purpose of the study was to determine the dynamics of morphometric changes in the lumen diameter of hemomicrocirculatory vessels in the retina of rats under normal conditions and under the combined influence of food additives – monosodium glutamate, sodium nitrite, and Ponceau 4R.

Materials and methods. The study involved 84 sexually mature male rats. Animals in the control group were given drinking water and received oral administration of physiological saline. Rats in the experimental group, with free access to water, were orally administered a combination of food additives once daily: sodium nitrite at a dose of 0.6 mg/kg, monosodium glutamate at 20 mg/kg, and Ponceau 4R at 5 mg/kg, all dissolved in 0.5 ml of distilled water. These doses were half of the maximum permissible limits. Adaptive behavior of the rats was assessed using the open field test. Animals were euthanized at 1, 4, 8, 12, and 16 weeks by an overdose of thiopental anesthesia. After euthanasia, fragments of retina were fixed in 10 % formalin. The tissue samples were then embedded in paraffin using standard histological procedures [8]. Sections of 5–10 μm thickness were prepared using an ARM 3600 microtome. After staining with hematoxylin and eosin, the sections were mounted in polystyrene and reviewed under a light microscope. Microphotography and morphometric analysis were performed using a Levenhuk D740T digital microscope equipped with a digital photoadapter and software adapted for this study. Statistical analysis of the morphometric data was conducted using Microsoft Excel with the built-in "Analysis ToolPak – VGA" add-on, specifically the "Descriptive Statistics" tool. The Shapiro–Wilk test was used to assess normality of the data distribution, and for normally distributed variables, comparisons were made using the Student's t-test for independent samples. Differences were considered statistically significant at $p<0.05$ [6].

All animal experiments were carried out in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (Strasbourg, 1986), in accordance with the rules for keeping experimental animals established by European Parliament and Council Directive (2010/63/EU) and the Order No. 134 of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012, No. 249 "On approval of the procedure for conducting tests, experiments on animals by research institutions", as well as the recommendations of the First National Congress of Ukraine on Bioethics (2001).

Results of the study and their discussion. The conducted morphometric study established that the average diameter of the lumen of arterioles in rats of the control group was 21.5±0.47 μm. The hemomicrovessel wall had a classical structure. After a week of the experiment, the diameter of the lumen of the resistive link had decreased significantly by 10.23 % to 19.3±0.51 μm ($p<0.05$) (Table 1).

Table 1

Morphometric parameters of the lumen diameter of hemomicrocirculatory vessels of rat's retina (μm)

Week	Arteriolar lumen diameter	Venule lumen diameter	Capillary lumen diameter
Control	21.5±0.47	37.1±0.34	5.5±0.15
1	19.3±0.51 *	35.2±0.26 *	3.3±0.12 *
4	15.1±0.56 *,**	31.8±0.21 *,**	4.2±0.11 *,**
8	22.6±0.46 *,**	34.3±0.33 *,**	5.7±0.09 **
12	24.3±0.37 *,**	36.8±0.39 **	6.3±0.11 *,**
16	24.1±0.38 *	40.5±0.41 *,**	6.8±0.13 *,**

Notes: * – $p<0.05$ compared to the control group; ** – $p<0.05$ compared to the previous observation period.

By the fourth week of the experiment, the diameter of the arteriole lumen continued to decrease, reaching a value of 15.1±0.56 μm, which was 29.77 % less than that of the control group and 21.6 % less than the previous period of the experiment ($p<0.05$). At the eighth week of observation, restoration of blood perfusion in arterioles was observed, with values of 22.6±0.46 µm. They were 22.19 % higher than the previous period of the experiment and 5.12 % higher than the indicator in the control group of animals (p<0.05).

By the twelfth week, a further increase in the average diameter of the arteriole lumen was observed, which was 24.3±0.37 µm, which was 13.02 % greater than the control and 7.52 % greater than the previous period of the experiment (p<0.05). At the sixteenth week of observation of experimental animals, the arterioles remained consistently dilated, with an average lumen diameter of 24.1±0.38 µm, which was 12.09 % significantly greater than the control values (p<0.05). However, this value did not differ significantly from those of the previous period of the experiment.

In animals of the control group, the average diameter of the lumen of the capacitive link was 37.1±0.34 µm. In the first week of the experiment, the average diameter of the venule lumen decreased significantly by 5.12 % to 35.2±0.26 µm (p<0.05). By the fourth week, the diameter of the venule lumen had significantly decreased both compared to control values (by 14.29 %) and compared to values in the previous group of animals (by 9.66 %) (p<0.05).

At the eighth week of observation, the diameter of the venule lumen significantly increased by 7.86 % compared to the values in the previous period and reached 34.3±0.33 µm, but was significantly smaller by 7.55 % compared to the control group of animals (p<0.05).

At the twelfth week of the experiment, the values increased significantly by 7.29 % (p<0.05) compared to the previous period, which was 36.8±0.39 microns. This increase did not differ significantly from the indicator in the control group. By the sixteenth week, the average diameter of the venule lumen had further increased and reached 40.5±0.41 µm. The indicator was 9.16 % higher than the control group of animals and 10.5 % higher than the value at the previous observation period (p<0.05).

The average diameter of the capillary lumen in the retina of the control group of rats is 5.5±0.15 µm. After a week of using the complex of food additives, the indicator decreased by 40.0 %, compared to the control group of animals, and was 3.3±0.12 µm (p<0.05).

After four weeks of observation, there was a tendency to restore blood perfusion through the exchange link of the retinal hemomicrocirculatory bed, which was manifested by an increase in the lumen diameter to 4.2±0.11 µm, which was 23.64 % significantly higher than the value in the control group and 27.27 % higher than the previous observation period (p<0.05). At the eighth week, the trend towards an increase in capillary lumen diameter values persisted and was accompanied by a restoration of the indicator to values in the control group. Subsequently, by the end of the observation, a gradual increase of approximately 10 % was determined, compared to the previous period of the experiment.

Thus, the conducted morphometric study established typical reactive changes in the hemomicrocirculatory system linked to the action of a complex of food additives, which are consistent with the results of other researchers [9, 12].

Conclusion

The effect of the complex of nutritional supplements on the resistive link of the hemomicrocirculatory bed is manifested by spasm due to activation of NMDA receptors at week 1, partial compensation due to angiogenesis at week 4, and remodeling of fibrous tissue and loss of elasticity at week 12–16. Regarding the capacitive link, spasm is determined in the early stages, while dilation occurs in the later stages. In the exchange link, ischemic spasm was detected in the early stages of the experiment, endothelial desquamation occurred in the eighth week, and pericyte loss and microaneurysm formation were observed in the 12th to 16th week.

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EXPERIMENTAL EVALUATION OF A THERAPEUTIC-PROPHYLACTIC COMPLEX ON BIOCHEMICAL MARKERS IN RAT GINGIVAL HOMOGENATES UNDER MODELLED FLUOROSIS AND ORTHODONTIC INTERVENTION

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The study was devoted to evaluate the effect of a therapeutic-prophylactic complex on inflammatory and oxidative-stress biomarkers in gingival homogenates of rats exposed to chronic excessive fluoride intake and orthodontic tooth movement. Experimental studies were carried out on 40 male Wistar rats (herd breeding), 4 months of age, with an average body mass of 280±14 g. Summarising the biochemical findings, orthodontic ligature fixation superimposed on fluorosis markedly disrupted the antioxidant-prooxidant balance and intensified inflammatory processes in the gingival tissues. Administration of the therapeutic-prophylactic complex under conditions of experimental fluorosis and orthodontic intervention improved antioxidant status, decreased lipid peroxidation, and attenuated inflammation, thereby confirming the antioxidant and anti-inflammatory properties of the agents incorporated into the developed therapeutic-prophylactic complex.

Key words: fluoride, fluorosis, gingiva, rats, experimental study.

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ЕКСПЕРИМЕНТАЛЬНА ОЦІНКА ВПЛИВУ ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНОГО КОМПЛЕКСУ НА БІОХІМІЧНІ МАРКЕРИ У ГОМОГЕНАТАХ ЯСЕН ЩУРІВ НА ТЛІ МОДЕЛЮВАННЯ ФЛЮОРОЗУ ТА ОРТОДОНТИЧНОГО ВТРУЧАННЯ

Дослідження присвячене визначенню впливу лікувально-профілактичного комплексу на біохімічні маркери запалення та оксидативного стресу у гомогенатах ясен щурів за умов хронічного надлишкового надходження фтору та ортодонтичного переміщення зубів. Експериментальні дослідження проведені на 40 щурах-самцях лінії Вістар (стадне розведення) віком 4 місяці, середньою масою тіла 280±14 г. Узагальнюючи результати біохімічних досліджень тканин ясен дослідних щурів можна відмітити, що фіксація ортодонтичної лігатури на тлі флюорозу негативно діє на антиоксидантно-прооксидантну систему та призводить до посилення запальних процесів у яснах тварин. Застосування лікувально-профілактичного комплексу препаратів у яснах щурів при експериментальному флюорозі та ортодонтичному втручанні призводить до покращення стану антиоксидантної системи, зниження рівня перекисного окислення ліпідів та інтенсивності запалення, що підтверджує наявність антиоксидантної та протизапальної дії препаратів що входять до розробленого лікувально-профілактичного комплексу.

Ключові слова: фтор, флюороз, ясна, щури, експеримент.

The work is a fragment of the research project "Development and introduction into clinical practice of methods of diagnosis, prevention and treatment of osteogenesis disorders during dental intervention in patients in wartime", state registration No. 0123U103247.

Gingival inflammation and dental malocclusions are common conditions in adolescents that can significantly compromise oral hygiene and overall periodontal health. Periodontal disease remains a highly prevalent disorder worldwide (with severe periodontitis affecting roughly 10 % of the population) [8]. Orthodontic appliances (fixed braces) exacerbate this problem by creating additional plaque-retentive