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REMODELLING OF MORPHOLOGICAL AND FUNCTIONAL PARAMETERS OF THE GUMS UNDER THE INFLUENCE OF FOOD ADDITIVES COMPLEX

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Currently, food additives are widely used in the food industry. This is not only because they significantly enhance the taste of products but also because they extend their shelf life. However, their negative effects often become apparent only after sometime. This experiment aimed to examine the impact of various food additives on the gums and changes in their composition. The study revealed that a combination of food additives (monosodium glutamate, sodium nitrite, and Ponceau 4R) affects the microvasculature of rat gums, leading to hemodynamic disturbances and, over time, impairing blood supply to the gums. Morphological changes in microvessels were mainly observed from the fourth week of the experiment, characterized by vasodilation or vasoconstriction and the formation of erythrocyte aggregates. These changes resulted in significant pathological alterations in the structural organization of the gums and their bloods upply, affecting the oral cavity as a whole.

Key words: oral cavity, pathological changes, gums, mucous membrane, lamina propria, morphometry, periodontium, connective tissue.

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РЕМОДЕЛЮВАННЯ МОРФОФУНКЦІОНАЛЬНИХ ПОКАЗНИКІВ ЯСЕН ПІД ДІЄЮ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК

На сьогоднішній день харчові добавки широко використовуються в харчовій промисловості. Це пов'язано не лише з тим, що вони значно покращують смак продуктів, але й подовжують термін їх зберігання. Однак їхній негативний вплив часто стає очевидним лише через деякий час. Цей експеримент мав на меті дослідити вплив різних харчових добавок на ясна та зміни в їхньому складі. Дослідження показало, що комбінація харчових добавок (глутамат натрію, нітрит натрію та Ponceau 4R) впливає на мікросудини ясен щурів, що призводить до гемодинамічних порушень і з часом погіршує кровопостачання ясен. Морфологічні зміни в мікросудинах спостерігалися переважно з четвертого тижня експерименту і характеризувалися вазодилатацією або вазоконстрикцією та утворенням агрегатів еритроцитів. Ці зміни призводили до значних патологічних змін у структурній організації ясен та їх кровопостачанні, впливаючи на стан ротової порожнини в цілому.

Ключові слова: ротова порожнина, патологічні зміни, ясна, слизова оболонка, власна пластинка, морфометрія, пародонт, сполучна тканина.

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In today's world, where the need for food is growing, manufacturers use various methods to speed up production and extend the shelf life of products. One of these methods is the use of chemicals. Some food additives have seemingly beneficial properties, such as preventing food spoilage, improving its taste and appearance, but the usefulness or harmfulness of their effects is highly controversial and poorly understood [1]. Their content in food products is strictly controlled by both national legislation and international standards. The use of certain additives may be allowed in one country but prohibited in another [4, 5].

However, even if the level of chemical additives does not exceed the permissible limits, their regular consumption can cause allergic reactions and pathological changes in the body. Studies conducted at the University of Southampton (UK) on the chronic and acute effects of artificial colors and preservatives on the behavior of more than 130 children (the mixture included the preservative sodium benzoate and dyes, including Ponceau 4R) showed that mixtures of this composition can affect the behavioral reactions of children [6].

Of course, our body has a defense mechanism. A significant part of food additives of any nature is excreted from the body after oxidation, reduction, hydrolysis and conjugation processes, which occur mainly in the liver, and therefore, the liver is one of the main organs that suffer from ingestion of a significant amount of food additives or their combinations [8–10].

Today, there are many scientific papers analyzing the effects of individual supplements, but research on their combined effects remains limited.

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Due to the lack of scientific trials in this area, the issue of studying morphological and functional changes in the body with the daily consumption of several food additives, even in permissible doses, is extremely relevant. This is an important issue for public health not only within our country but also around the world.

The purpose of the study was to determine the morphological and morphometric changes in the gums of rats under the influence of a complex of food additives in the experiment.

Materials and methods. The study was conducted on white rats weighing 0.350±0.15 g, 6–8 months old, which were kept in standard conditions of the vivarium of Poltava State Medical University. They were kept in standard cages (90cm (L) x 60cm (D) x 120cm (H), 4 rats/cage, with corn cob bedding. In cage they had 3 cardboard tunnels and nesting material from wood and wool. Rats had a controlled temperature in g=cages (22±2°C), middle humidity (50±10%) and 12 hours light/dark cycle. Experiment last for half a year from March 2024 to September of 2024. The experimental studies were conducted in compliance with the biotic requirements and humane treatment of experimental animals regulated by the Law of Ukraine "On the Protection of Animals from Cruelty" (No. 3447-IV of February 21, 2006) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

The animals were divided into 6 groups (1 control group (n=15) and 5 experimental groups (n=75)). In the control group, rats were orally administered saline, with free access to drinking water. Rats from the experimental groups were orally administered at the same time a mixture of 10 % sodium nitrite solution (E 250), sodium glutamate (E621) at a dose of 20 mg/kg in 0.5 ml of distilled water, Ponceau 4R at a dose of 5 mg/kg in 0.5 ml of distilled water once a day, also under the condition of free access to drinking water. The dosage of food additives was half that of the permissible norm of food additives in food products as defined by the legislation of Ukraine "On Standardization". The rats were withdrawn from the experiment after 1, 4, 8, 12 and 16 weeks by an overdose of thiopental anesthesia (30 mg, KYIVMEDPREPARAT). All rats survived to the end of experiment. Then, gingival fragments fixed in neutral formalin solution were embedded in paraffin.

The histological sections made from paraffin blocks were stained with hematoxylin and eosin for the overview sections. After examining the overview sections, immunohistochemical reactions were performed on them, and then microscopy was performed using a light microscope Viorhex with a digital microphotomount DSM 900. To obtain semi-thin and ultra-thin sections, the study material was fixed in glutaraldehyde in phosphate buffer and sealed in EPON-812. The finished sections were stained with toluidine blue and polychrome dye. For electron microscopic examination, epoxy blocks were used to make ultrathin sections on an ultramicrotome, which were then placed on copper grids and contrasted with a 1 % aqueous solution of uranyl acetate and lead citrate according to the Reynoldsase method and examined using a PEM-125K electron microscope.

During the research, we used a system for visual analysis of histological specimens. The images were displayed using a microscope and a Vision CCD camera. Morphometric studies were performed using the programs VideoTest-5.0, KAAPA ImageBaseta Microsoft Excel. We determined the thickness of the gingival epithelium and lamina propria, and then processed the data using Statistica 10 BiostatPro 6 software and Microsoft Excel 2019. Verification of calculations and indicators in the samples was performed using the Shapiro-Wilk test. Quantitative data evaluation included the determination of the arithmetic mean of the variation series (M) and its standard error (m). To compare quantitative values in paired rows, we used Student's t-test. The difference was considered significant at $p<0.05$.

Results of the study and their discussion. Morphological analysis showed that the gums of the rats in the control group were covered with a mucous membrane without a submucosal layer. It was directly connected to the gingival plate, and its surface layer formed high narrow papillae that penetrated the epithelial layer of the mucous membrane. The epithelium itself consisted of a multilayered squamous epithelium (Fig. 1).

The surface layer of epithelial cells in areas of keratinization is called stratified, and in places where this process does not usually occur, the surface is represented by a layer of squamous cells. Below the stratum corneum is a granular layer, the cells of which are elongated and contain keratohyaline grains. In areas without keratinization, there are several rows of spindly polygonal cells under the flat layer.

The deepest layer of the epithelium is the basal layer, which consists of cylindrical or cubic cells arranged in a single row and adjacent to the basement membrane. It is this layer that provides epithelial renewal.

Using the morphometric method, it was found that the average thickness of the epithelial plate of the attached part of the gums of the control group rats is 61.15±5.43 microns, its own 120.38–11.35 microns.

After 1 week of the experiment, the mean values of the thickness of the epithelial plate significantly decreased by 27.2 % to 44.52±5.94 microns ($p<0.05$). The thickness of the own plate of the attached part of the gingiva in rats increased by 58.2 % compared to the values in the control group of rats to 190.42±6.12 microns ($p<0.05$) due to edema and, accordingly, an increase in the amount of amorphous substance.

On the fourth week of the experiment, the average thickness of the epithelial laminae increased slightly compared to the previous period and amounted to 54.12±6.32 μm.

As for the thickness of the lamina propria, there was a tendency to its decrease compared to the previous period (173.6±7.81 μm), but this indicator remained significantly higher by 44.2 % ($p<0.05$) compared to control animals (Fig. 2).

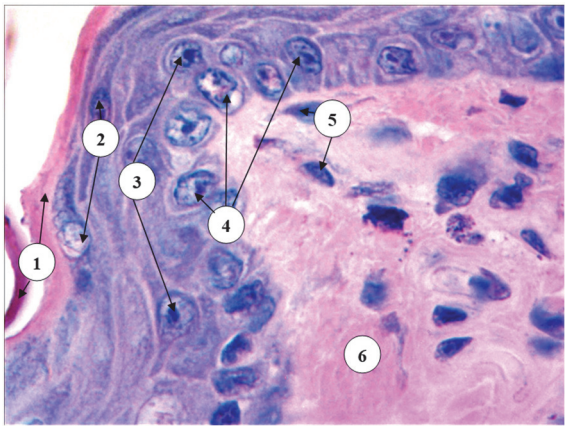

Fig. 1. Morphological structure of the attached part of the gums of rats of the control group. Staining with polychrome dye. Collection: Ok. 10, Ob. 100. Note: 1 – horny scales; 2 – nuclei of granular layer cells; 3 – cells of spinous layer; 4 – basal cells; 5 – fibroblasts; 6 – collagen fibers.

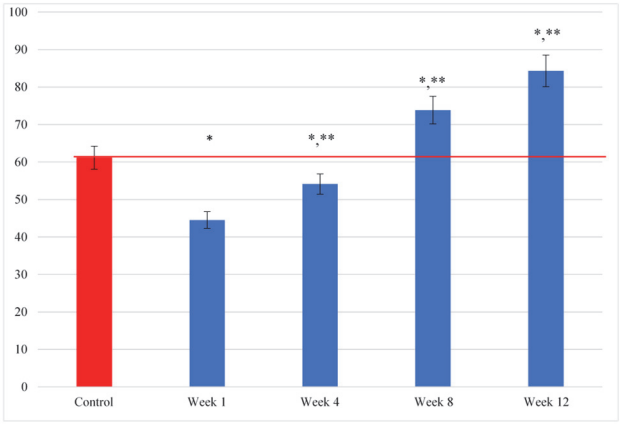

Fig. 2. Changes in the thickness of the gingival mucosa epithelium in rats in the dynamics of the experiment. Note: * – statistically significant at $p<0.05$ compared to the control group, ** – statistically significant at $p<0.05$ compared to the control group, statistically significant at $p<0.05$ compared to the previous observation period.

After the eighth week of the experiment and the use of food additives, the thickness of the epithelial plate of the mucous membrane of the attached part of the gums increased to 73.85±6.62 μm, which was significantly more by 36.4 % compared to the previous period ($p<0.05$), but the difference with control rats remained statistically insignificant.

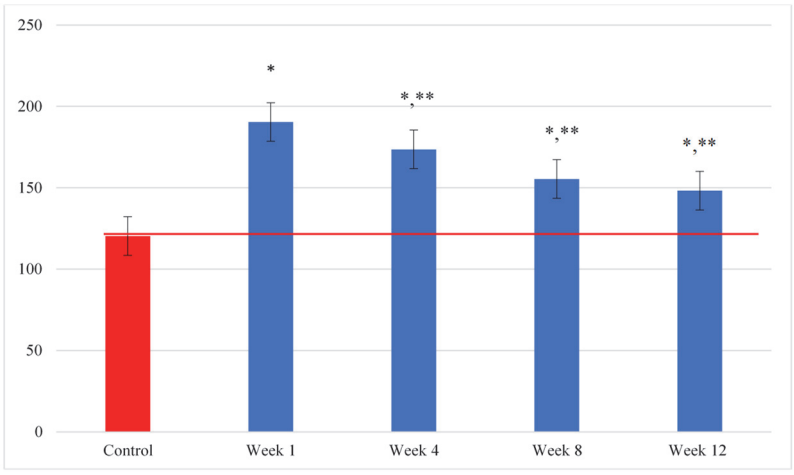

Fig. 3. Changes in the thickness of the gingival mucosa of rats in the dynamics of the experiment. Note: * – statistically significant at $p<0.05$ compared to the control group, ** – statistically significant at $p<0.05$ compared to the control group, statistically significant at $p<0.05$ compared to the previous observation period.

The average thickness of the own plate maintained a downward trend and reached 155.31±6.33 μm, but was 29 % higher than the value in the control group of rats.

By the 12th week of the experiment, the epithelial lamina continued to thicken: the average value was slightly higher than the previous one ($p<0.05$), and significantly exceeded the control value by 37.9 %.

Regarding the average thickness of the lamina propria, a slight decrease was observed, but it remained 23 % higher compared to the control group of rats (Fig. 3).

Under the influence of a complex of food additives during the first week, a decrease in the number of cell layers in the basal layer was found, but these changes were not significant and the index remained at the level of 1.25±0.43.

In the spinous and granular layers, there was a significant decrease in the average number of cell layers: in the spinous layer – from 3.61±0.011 to 2.45 0.05, and in the granular layer – from 3.1±0.04 to

1.7±0.06 ($p<0.05$). At the same time, an increase in the average number of cell layers was observed in the stratum corneum to 3.12±0.04 ($p<0.05$).

By the 4th week of the experiment, the average number of cell layers in the basal layer continued to decrease (from 1.12±0.41 at week 1 to 1.03±0.03), indicating inhibition of the proliferative activity of keratinocytes. However, it was significantly different from the values in the control group of rats (1.11±0.03 and 0.9±0.02, respectively ($p<0.05$).

The average number of cell layers in the spiny layer increased compared to the previous period of the experiment and exceeded the index in the control group of animals ($p<0.05$).

The average number of cell layers in the granular layer increased from 1.7±0.05 to 2.85±0.14 ($p<0.05$), but was less than the value in the control group of rats ($p<0.05$).

In the stratum corneum, a progressive decrease in the average number of cell layers was observed from 3.26±0.04 to 2.1±0.06, in the control group – to 2.68±0.11 ($p<0.05$), which is a morphological confirmation of increased desquamative processes in the epithelium of the attached part of the gums.

After 8 weeks from the beginning of the experiment, the average number of cell layers decreased, but did not differ significantly from the control group. The average number of cell layers in the spiny layer decreased significantly.

At the 12th week of the experiment, the average number of cell layers in the basal layer of the epithelial plate of the attached part of the gingiva decreased slightly, but did not differ significantly from the control group of animals.

Compared with the values in the control group of animals, the average number of layers of cells of the granular layer exceeded the value for the previous period

After finishing the experimental modeling of the effect of a complex of food additives on the gingival mucosa of rats, we have established regular reactions and structural changes in the gums.

It is noteworthy that after 1 week of the experimental study, the thickness of the epithelium decreases significantly, statistically significant, at $p<0.05$. At the same time, the lamina propria, on the contrary, increases its thickness. Due to this process, there is a corresponding disruption of hemodynamic processes.

After 4 weeks of the experimental study, the average thickness of the epithelium and lamina propria changed in response to the restorative-compensatory reactions aimed at neutralizing the source of alteration and restoring the morphofunctional state of the surrounding microvessels. However, these processes do not fully lead to a complete restoration of normal indicators compared to similar indicators of the control group of animals, which indicates irreversible changes in the state of the loose connective tissue of the gingival mucosa of rats, and this in turn leads to decompensation of these processes, which in turn is manifested by signs of hypoxia [3].

After 12 weeks of observation, the morphometric parameters are not restored to similar indicators of the control group of animals. At the microscopic level, in the deep layers of the gingival mucosa, numerous groups of mastocytes in the stage of degranulation and in the stage of accumulation of secretory granules are visualized. In our opinion, mastocytes play a significant role in restoring the local epithelial condition to the control group due to degranulation of secretory granules containing heparin. Particularly noteworthy is the fact of changes in the rheological properties of blood with the formation of wall dextrin slides of erythrocytes, which, in our opinion, is associated with the entry into the systemic bloodstream of food additives containing glutamate and sodium nitrite and causing both allergic reactions and subsequent systemic inflammatory processes [4, 7]. The revealed changes in the metric parameters of the structural components of the rats' gastric fundus wall of rats after the consumption of glutamate, sodium nitrite and Ponceau 4R are primarily caused by their direct effect on the surface of the gastric mucosa, which leads to alteration and exudation and is stereotyped for many aggressive factors [11]. Despite the use of statistical methods, the small sample size (N=75 rats) may have affected the level of statistical significance. For more accurate data, it would be preferable to use a larger number of rats in the next study.

Conclusion

Prolonged use of a complex of food additives containing monosodium glutamate, sodium nitrite and Ponceau 4R dye leads to irreversible disorders of hemodynamics of the gingival mucosa, causing hypoxic changes in the surrounding tissues.

Uneven thinning or thickening of the epithelial layer and the lamina propria, accompanied by destructive changes at the ultrastructural level and the formation of parietal dextrin slides of red blood cells, leads to a violation of metabolic processes in the tissue as a whole.

Recovery and compensatory reactions aimed at eliminating the source of damage and normalizing the morphological and functional state of tissues do not ensure their complete recovery. This is manifested at the later stages of the study in the form of an increase in the thickness of the epithelium and the lamina propria, which indicates decompensation of the recovery processes in the gingival mucosa.

The presence of numerous groups of mastocytes in the deep layers of the mucous membrane, both in the phase of degranulation and in the stage of accumulation of secretory granules, indicates the restoration of local hemodynamics. This process occurs due to the release of heparin from secretory granules.

Our study not only confirms the negative impact of food additives on the body, but also demonstrates their complex effect. Unlike other studies that focus on the effects of individual substances, our experiment shows the effects of the simultaneous use of several additives. This is important because most foods contain combinations of different additives. Thus, the results are valuable for both basic research and practical medicine, as they allow us to trace the time sequence of changes in tissues and develop effective approaches to their correction.

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