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

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Nowadays, food additives are widespread in the food industry. This is not only due to the fact that they greatly improve the taste of products but also because they allow products to be stored for a longer period of time. Unfortunately, they have many negative properties that manifest themselves only after a certain period of time. The experiment aimed to identify the effects of various food additives on the gums and changes in their composition. The study found that the impact of a complex of food additives (monosodium glutamate, sodium nitrite and Ponceau 4R) on the microvasculature of the gums of rats causes a violation of haemodynamic conditions, and over time a violation of the blood supply to the gums. Morphological changes of microvessels are observed mainly from the fourth week of the experiment and are characterised by vasodilation or vasoconstriction, the formation of erythrocyte aggregates, which leads to significant pathological changes in the structural organisation of the gums and blood supply to the gums as an integral organ of the oral cavity.

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

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РЕМОДЕЛЮВАННЯ МОРФОФУНКЦІОНАЛЬНИХ ПОКАЗНИКІВ ЯСЕН ТА ЕЛЕМЕНТІВ ГЕМОМІКРОЦИРКУЛЯТОРНОГО РУСЛА ПІД ДІЄЮ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК

В наші часи харчові добавки широко поширені в харчовій промисловості. Це пов'язано не тільки з тим, що вони сильно поліпшують смаковівластивості продуктів, але і тому, що вони дозволяють зберігати продукти протягом більш тривалого періоду часу. На жаль, вони мають безліч негативних властивостей, які проявляються лишечерез певний період. Експеримент мав на меті виявити вплив різних харчових добавок на ясна та зміни їх складу. У ході дослідження було виявлено, що дія комплексу харчових добавок (глутамат натрію, нітрит натрію та Понсо 4R) на мікросудини ясен щурів викликає порушення гемодинамічних умов, а з часом – порушення кровозабезпечення ясен. Морфологічні зміни мікросудин спостерігаюся переважно із четвертого тижня експерименту і характеризуються вазодилятацією або вазоконстрикцією, утворенню еритроцитарних агрегатів, що призводить до суттєвих патологічних змін у структурній організації ясен та кровозабезпечення ясен, як цілісного органа ротової порожнини.

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

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With the ever-increasing demand for food, manufacturers are using various methods to speed up production and increase the shelf life of their products. The use of various chemicals, including some food additives, is one of the ways for industrialists to achieve these goals. Some food additives have seemingly beneficial properties, such as preventing food spoilage and improving its taste and appearance, but the usefulness or harmfulness of their effects is highly debatable and poorly understood [6]. It's crucial for consumers to understand these effects to make informed choices about their food. However, a large number of food additives pose a threat to human health[4]. The amount of food they produce is strictly regulated by both domestic and international legislation. The harmlessness of food additives is determined on the basis of extensive comparative studies, and the use of food additives is prohibited unless they have been tested and approved by the relevant authorities [1].

The human body's response to food additives is highly individual. The fact that monosodium glutamate (E621) can have pathophysiological and toxicological effects on human health has been proven by more than one study [3]. Especially in people who are sensitive to monosodium glutamate, headaches, urticaria, and bronchial asthma attacks can occur [2].

To date, scientific literature has been found that discusses the effects of using different food additives individually, but there has been little research into changes in the body when several additives are used together. A very limited number of scientific trials have been carried out in this area. One study in which two preservatives were tested together showed that they have a much stronger effect in combination than separately. Or another example. According to Harvard Health Publishing, artificial food colours increase the risk of attention deficit hyperactivity disorder in children [9].

Therefore, the problem of studying morphological and functional changes in the body due to daily consumption of many food additives, even in the maximum permissible amount, is an extremely relevant and important issue for ensuring public health in our country and humanity as a whole.

The purpose of the study was to determine the morphological and morphometric changes in the structural components of the venules of the haemomicrocirculatory channel of the gingival mucosa of rats under the influence of a complex of food additives in the experiment.

Materials and methods. The study was carried out on white rats weighing 0.350±0.15 g, which were kept in standard conditions of the vivarium of Poltava State Medical University. 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 21.02.2006) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

The animals were divided into six groups (1 control group (n=15) and five 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), monosodium 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 with 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 Standardisation'. The rats were withdrawn from the experiment after 1, 4, 8, 12 and 16 weeks by an overdose of thiopental anaesthesia. Gingival fragments fixed in a neutral formalin solution were then embedded in paraffin. Histological sections made from paraffin blocks were stained with haematoxylin and eosin for the examination sections. After examining the sections, immunohistochemical reactions were performed on them, followed by microscopy using a Viorhex light microscope with a digital microphotographic nozzle DSM 900. The study material was fixed in glutaraldehyde in phosphate buffer and sealed in EPON-812 to obtain semi-thin and ultra-thin sections. 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 support grids and contrasted with a 1 % aqueous solution of uranyl acetate and lead citrate according to the Reynolds method with modifications and examined using a PEM-125K electron microscope. The average total diameter of arterioles, as well as their average lumen diameter and vascular wall thickness in the gingival mucosa of rats, were determined using the morphometric method. When conducting morphological and morphometric studies, we used a system for visual analysis of histological sections. The images were displayed using a microscope and a Vision CCD camera. Morphometric studies were performed using VideoTest-5.0, KAAPA ImageBaseta Microsoft Excel software. We determined the total average diameter, average lumen diameter, and wall thickness of arterioles, venules, and capillaries and processed the data using Statistica 10 BiostatPro 6 software and Microsoft Excel 2019. The calculations and parameters in the samples were checked 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. The morphological examination revealed that the gums of the control group rats were covered with a mucous membrane with no submucosal base. The mucous membrane was directly connected with its lamina propria, and its surface layer formed high, narrow papillae that grew into the epithelial layer of the mucous membrane. A multilayered squamous stratified epithelium represented the epithelial layer. Elements of the hemomicrocirculatory system were visualised both directly in the lamina propria and its papillae and penetrated into the deeper parts of the epithelial layer. Namely, we studied venules as an exchange link of the hemomicrocirculatory bed. Venules were defined as small, rounded structures that consisted of three membranes: inner, middle, and outer. The inner layer was represented by endothelial cells located on the inner elastic membrane, which had a tortuous course. The middle layer was visualised as a gap formed by 1 or 2 layers of smooth myocytes. The outer layer was represented by a thin layer of loose connective tissue. Venules are generally thinner and have less vessel wall area than arterioles[7].

The haemomicrocirculatory bed of the gingival mucosa of rats is extremely developed. Venules, as an exchange link of the microcirculatory bed in the dynamics of the experiment, reacted to the introduction of food additives by vasodilation and vasoconstriction, as well as by thinning or thickening of the vascular wall.

Thus, the average total diameter of venules decreased by 4.77 % at week 1 of the experiment at $p<0.05$ compared to the control values. During weeks 4 and 8 of the experiment, the average total diameter of venules was restored to the control values and even increased by 15.4 % at week 8. At the 12th week of observation, this indicator decreased significantly compared to the 8th week, but by the 16th week, it increased by 18.87 % compared to the control values (Fig. 1).

In addition to changes in the average total diameter of venules, there were functional changes in the average diameters of their lumens. Thus, after one week of taking the food additives complex, the average

diameter of the venular lumens decreased by 4.87 % compared to the control values. After four weeks of the experiment, this indicator was 2.04 % compared to the control group's. After the 8th week of observation, this indicator increased by 1.2 % compared to the control group. After 12 weeks of the experimental study, the average diameter of the venular lumen significantly increased by 2.38 % at p<0.05 compared with the control values, and the maximum value was reached after 16 weeks, showing an increase of 18.8 % (Fig. 2).

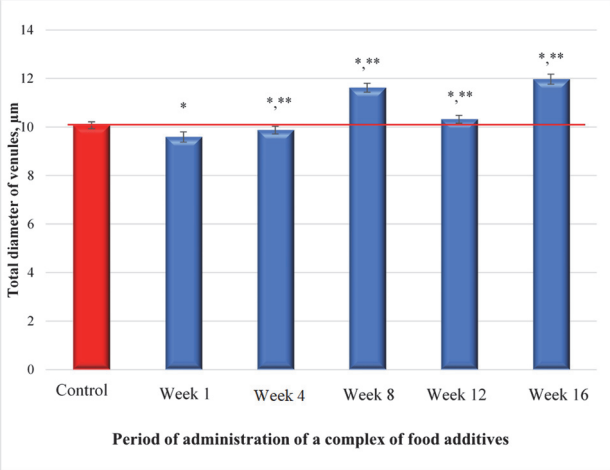

Fig. 1. Changes in the average values of the total diameter of the venules of the gingival mucosa of rats in the dynamics of the experiment.

Note: * – statistically significant at p<0.05 compared to the control group, ** – statistically significant at p<0.05 compared to the previous observation period.

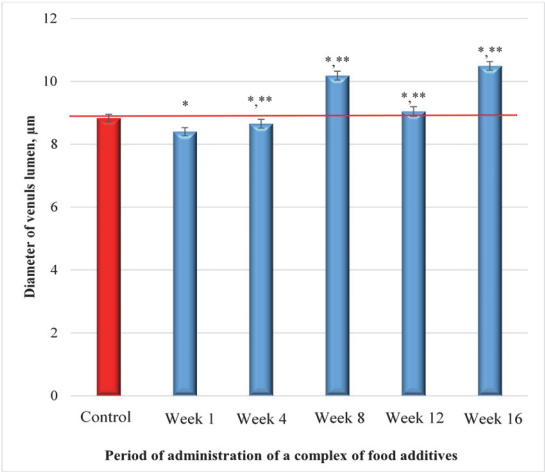

Fig. 2. Changes in the mean diameters of the lumen of the venules of the gingival mucosa in rats in the dynamics of the experiment.

Note: * – statistically significant at p<0.05 compared to the control group, ** – statistically significant at p<0.05 compared to the previous observation period.

A detailed analysis of the structural components of the vascular wall of the venules showed that after 1 week of taking the food additives complex, the vascular wall significantly thinned by 69.9 % at p<0.05 compared to the control values. At the ultramicroscopic level, it was determined that the venular wall underwent significant destructive changes. In the inner layer of the arterioles, endothelial cells became flattened, and some lost their connection with the basement membrane. In contrast to such structures in the control group of animals, the inner elastic membrane lost its tortuous course. In the middle layer, smooth myocytes were visualised as thinned and elongated; in some places, they had no connection with the elastic membrane. In the outer layer, the layer of loose connective tissue was significantly thinner compared to the venules of the gingival mucosa of the control group of animals.

As with the previous indices, the study showed that from the beginning of the experiment to week 4, the vascular wall thickening of the venules decreased by 4.1 % and 1.61 %, respectively, compared to the control group. At 8 weeks of observation, a 16.13 % thickening of the vascular wall was detected, which was significant at p<0.05. At this time, at the microscopic level, restoration of the organisational structure of the inner venous layer was detected. After 16 weeks of the experimental study, there was a further significant (p<0.05) thickening of the vascular wall by 11.6 % due to swelling of the layer of loose connective tissue of the outer venous layer (Fig. 3).

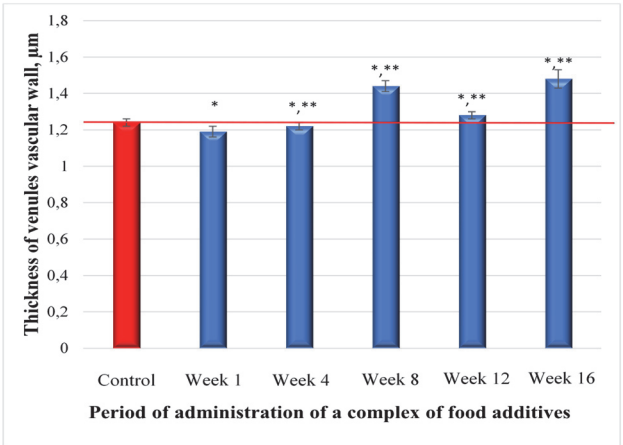

Fig. 3. Changes in the average thickness of the vascular wall of the venules of the gingival mucosa in rats in the dynamics of the experiment.

Note: * – statistically significant at p<0.05 compared to the control group, ** – statistically significant at p<0.05 compared to the previous observation period.

After experimental modelling of the effect of a complex of food additives on the gingival mucosa of rats, we have established regular reactions of venules, their structural changes and changes in the rheological properties of blood. According to the literature, the response of microvessels to the influence of environmental and internal environmental factors is quite specific and diverse. During the experiment we determined changes in the venules of the gingival mucosa of experimental animals. Notably, after one week of the experimental study, the mean total diameter of venules decreased significantly, statistically significantly, at p<0.05. At the same time, histological sections of the connective tissue surrounding the microvessel revealed hyperhydration of its amorphous

substance. Due to this process, venous compression is observed, and haemodynamic processes are disturbed. Also, a significant thinning of the vascular wall and the resulting changes in its structural organisation are clearly visible. Particularly noteworthy is the fact of changes in the rheological properties of blood with the formation of erythrocyte wall dextrin slides, which, in our opinion, is associated with the intake of food additives containing glutamate and sodium nitrite in the systemic bloodstream and causing both allergic reactions [5] and subsequent systemic inflammatory processes [8, 10].

After four weeks of the experimental study, the average values of the total diameter, lumen diameter and vascular wall thickness of venules changed in response to the restorative-compensatory reactions aimed at neutralising the source of alteration and restoring the morphological and functional state of the surrounding microvessels. However, these processes do not fully lead to a complete restoration of normal haemodynamic parameters compared to similar indicators of the control group of animals, as evidenced by an increase in the lumen of microvessels (including venules), the state of loose connective tissue of the gingival mucosa of rats, which in turn leads to decompensation of these processes, which in turn is manifested by signs of hypoxia.

After 16 weeks of investigation, the morphometric parameters do not recover 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 are visualised in the stage of degranulation and accumulation of secretory granules. In our opinion, mastocytes play a crucial role in the restoration of local hemodynamics by degranulating secretory granules containing heparin.

Conclusion

Prolonged intake of a complex of food additives, which includes monosodium glutamate, sodium nitrite and Ponceau 4R dye, in the body of rats causes irreversible processes of disturbance of the hemodynamics of the gingival mucosa with the development of hypoxic phenomena in the surrounding tissues.

Uneven thinning or thickening of the venous wall, which was a destructive change at the ultrastructural level with the formation of wall dextrin slides of erythrocytes, leads to changes in the rheological properties of blood and metabolic processes of all elements of the haemomicrocirculatory bed.

Restorative and compensatory reactions, which should be aimed at neutralising the source of alteration and restoring the morphological and functional state of blood vessels, do not lead to a complete restoration of normal haemodynamic parameters, which in the last weeks of the study is reflected in an increase in the average diameter of the venule and the average diameter of the lumen of other microvessels. It leads to the decompensation of recovery processes in the gingival mucosa.

Numerous groups of mastocytes in the deep layers of the gingival mucosa appear in the stage of degranulation and in the stage of accumulation of secretory granules, which indicates the cyclical nature of the processes of restoration of local hemodynamics. This is due to the breakdown of secretory granules containing heparin.

Our research not only confirms but clearly demonstrates the effects of food additives on the body. While other studies focus on the impact of a particular additive, our experiments show the characteristics of the complex effects created by several additives simultaneously. This is important because most of our products contain a mixture of food additives. Thus, our research is relevant for both theorists and practitioners, as it allows us to observe the time sequence of tissue changes and develop treatments accordingly.

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CHARACTERISTICS OF METRIC PARAMETERS OF THE ILEUM WALL IN RATS UNDER LONG-TERM COMPLEX INFLUENCE OF CHEMICAL FOOD ADDITIVES

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It has been experimentally proven that chemical food additives can cause changes in the morphological structure of various internal organs, including the digestive system. To date, data on the complex effects of these substances on the ileum are rather limited. It justified the aim of the study, which was to investigate changes in the intestinal wall after long-term administration of a complex of chemical food additives, including monosodium glutamate, sodium nitrite and ponceau 4R. In this study, using histological and morphometric methods of research, we determined the dynamic changes in the overall average wall thickness and its separate layers. It was found that the ileum of rats of both the control group (receiving saline) and the experimental groups (receiving a complex of chemical food substances for 1, 4, 8, 12, 16, and 20 weeks) is represented by serosa, muscular layer, submucosa and mucosa. It has been established that changes occur for up to 8 weeks due to hyperhydration of connective tissue and pericellular oedema of the structural components of all layers. However, starting from week 12, there are signs of irreversible changes that progress to week 20 and manifest as dystrophic changes with subsequent atrophy of the ileal wall, confirmed by a decrease in all indicators. Thus, with their prolonged administration, chemical food additives disrupt the protective and adaptive mechanisms of the ileum.

Key words: ileum, intestinal wall, food additives, morphometric analysis, rats, mucosa, serosa, lymphocytes.

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ХАРАКТЕРИСТИКА МЕТРИЧНИХ ПОКАЗНИКІВ СТІНКИ КЛУБОВОЇ КИШКИ ЩУРІВ ПРИ ТРИВАЛОМУ КОМПЛЕКСНОМУ ВПЛИВІ ХІМІЧНИХ ХАРЧОВИХ ДОБАВОК

Експериментально доведено, що хімічні харчові добавки можуть викликати зміни морфологічної будови різних внутрішніх органів, зокрема травної системи. На сьогодні, дані щодо комплексного впливу цих речовин на клубову кишку є досить обмеженими. Це й обґрунтувало мету дослідження, яка полягала у вивченні змін кишкової стінки при тривалому введенні комплексу хімічних харчових добавок, зокрема глутамату натрію, нітриту натрію та понсо 4R. У роботі, за допомогою гістологічного та морфометричного методів дослідження, було проведено визначення динамічних змін показників загальної середньої товщини стінки та окремих її оболонок. Було встановлено, що клубова кишка щурів як контрольної групи (отримувала фізіологічний розчин), так і експериментальних груп (отримували комплекс хімічних харчових речовин протягом 1, 4, 8, 12, 16, 20 тижнів) представлена серозною, м'язовою, підслизовою та слизовою оболонками. Встановлено, що до 8 тижня виникають зміни обумовлені гіпергідратацією сполучної тканини та перицелюлярним набряком структурних компонентів усіх оболонок. Але починаючи з 12 тижня з'являються прояви необоротних змін, які прогресують до 20 тижня та проявляються у вигляді дистрофічних змін з подальшою атрофією стінки клубової кишки, що підтверджується зменшенням всіх показників. Таким чином, хімічні харчові добавки при їх тривалому введенні порушують захисні та адаптаційні механізми клубової кишки.

Ключові слова: клубова кишка, кишкова стінка, харчові добавки, морфометричний аналіз, щури, слизова оболонка, серозна оболонка, лімфоцити.

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.

Dysfunctions of internal organs resulting from their morphological changes often occur due to the negative impact of endogenous and exogenous factors. Chemical food additives, widely used in the modern food industry, play an important role among the latter. They not only give food products a pleasant taste, smell, and attractive appearance but also prevent early spoilage, thereby extending the shelf life of food products. The results of studies show that these chemicals can cause changes in various internal organs [4, 5, 10]. However, given its direct contact with them, they have the most negative impact on the digestive