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MORPHOLOGICAL AND MORPHOMETRIC CHANGES IN CAPILLARIES OF THE HEMOMICROCIRCULATORY CHANNEL OF THE GINGIVAL MUCOSA DURING LONG-TERM EXPOSURE TO A COMPLEX OF FOOD ADDITIVES

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Food additives are an important element of the food industry in the 21st century, which not only improve the taste of products, but also increase their shelf life. At the same time, they have negative properties that manifest themselves after a long period of use. The purpose of the experiment was to identify the simultaneous effect of several food additives on the gums, their structure and the changes that occur in them. During the study, it was established that the effect of a complex of food additives (sodium glutamate, sodium nitrite, and Ponseau 4 R) on the vessels of the gums of rats causes a violation of hemodynamic conditions and irreversible violations of the blood supply to the gums. Morphological changes of the capillaries were observed throughout the experiment and are characterized by changes in the thickness of the walls and the diameter of the lumen, which leads to pathological changes in the structural gums and their blood supply.

That's a reason of these experiment. Understanding of mechanisms of influence of food additives will lead us to better treatment of different diseases and prevent of using such number of synthetic chemicals in food industry.

Key words: *oral cavity, pathological changes, gums, microcirculation, capillaries.*

Connection of the publication with planned research works.

This article was performed as part of research work: "Patterns of morphogenesis of organs, tissues and neurovascular formations in normal condition, pathology and under the influence of exogenous factors" (state registration № 0118U004457).

Introduction.

The 21st century is characterized by a sharp increase in the number of the population. There are already more than 8 million people on planet Earth, and this number will only grow. Accordingly, the need for food also increases. In addition, it is important to ensure long storage periods. The use of various chemical additives helps manufacturers in this. At first glance, some food additives have useful properties, for example, they prevent food spoilage, improve their taste and appearance, but their usefulness or harmfulness is a very controversial and poorly studied issue [1-3]. International and domestic legislation regulate the content of such substances. But, even if the permissible level of chemically created food additives is observed, their regular entry into the body can cause the appearance of allergic reactions, the emergence and development of pathological changes in the organs and systems of the human body [4].

The reaction of the human body to food additives is exclusively individual. At the moment, there is a large number of works that describe in detail the effect of a certain supplement on the body. At the same time, there are almost no data on the simultaneous effect of a complex of food additives. It is necessary to understand that products stably contain several additives at once in their composition, therefore it is necessary to study their joint influence. One study, in which two preservatives were tested together, showed that they have a much stronger effect in combination than individually [5]. This only confirms the importance of researching the effects of many food additives on the body when they are used every day. Even in acceptable amounts, they can cause health problems, let alone large doses. Therefore, the problem of food additives is urgent and important, ensuring the public health of the population not only of our country, but also of humanity as a whole.

The aim of the study.

To determine the morphological and morphometric changes in the structural components of the capillaries of the hemomicrocirculatory bed of the mucous membrane of the gums of rats under the influence of a complex of food additives in the experiment.

Object and research methods.

The study was conducted on white rats, weighing (0.350±0.15) g, which were kept under standard conditions in the vivarium of the Poltava State Medical University. Experimental studies were carried out in compliance with biotic requirements and humane treatment of experimental animals, regulated by the Law of Ukraine "On the Protection of Animals from Cruelty Treatment" (№ 3447-IV dated 21.02.2006) and the European Convention on the Protection of Vertebrate Animals Used for Research and other scientific goals (Strasbourg, 1986).

The animals were divided into 6 groups (1 control (n=15) and 5 experimental groups (n=75)). In the control group, rats were orally administered physiological saline, with free access to drinking water. Rats from the experimental groups orally, at the same time, were administered a mixture of a 10% solution of sodium nitrite (E 250), monosodium glutamate (E621) in a dose of 20 mg/kg in 0.5 ml of distilled water, Ponceau 4R – in a dose 5 mg/kg in 0.5 ml of distilled water once a day, also under the condition that animals have free access to drinking water. Dosages of food additives were twice less than their permissible rate in food products defined by the legislation of Ukraine "On Standardization". Rats were removed from the experiment after 1, 4, 8, 12 and 16 weeks by overdose of thiopental anesthesia.

Subsequently, the gingival fragments fixed in a neutral formalin solution were sealed in paraffin. Histological sections made from paraffin blocks were stained with hematoxylin and eosin for examination sections. After

studying the examination sections, immunohistochemical reactions were performed on them, and then microscopy using a Viorex light microscope with a DSM 900 digital photomicroscope. To obtain semi-thin and ultra-thin sections, the studied material was fixed in glutaraldehyde on a phosphate buffer and sealed in EPON-812. Ready sections were stained with toluidine blue and polychrome dye. For electron microscopic research, epoxy blocks were used, from which ultra-thin sections were made 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 changes [6] and studied using a PEM-125K electron microscope.

Using the morphometric method, the average total diameter of arterioles, as well as their average diameter of the lumen and the thickness of the vascular wall in the mucous membrane of the gums of rats were determined. When conducting morphological and morphometric studies, we used a system of visual analysis of histological preparations. We displayed the images using a microscope and a Vision CCD camera. Morphometric studies were carried out using the programs VideoTest-5.0, KAAPA ImageBase and Microsoft Excel. We determined the total mean diameter, the mean diameter of the lumen, and the wall thickness of arterioles, venules, and capillaries and processed the data using Statistica 10 BiostatPro 6 software, as well as using Microsoft Excel 2019. Verification of calculations and values in samples was performed using the Shapiro-Wilk test. Data quantification 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 $p<0.05$.

Research results and their discussion.

During the experimental study, it was found that the gums of rats in the control group were covered with a mucous membrane without a submucosal base. The mucous membrane is directly fused with its own plate, and its first layer was formed by tall and narrow papillae growing into the epithelial layer of the mucous membrane. The epithelial layer was represented by a multi-layered flat non-keratinized epithelium. The elements of the hemomicrocirculatory channel were visible both directly in the lamina propria and its papillae and penetrated into the deep parts of the epithelial layer, and the capillaries studied by us were defined as an exchange link of the hemomicrocirculatory channel. Capillaries were defined as structures with a small diameter and a rounded shape, which have a very thin wall formed by endothelial cells, a basal membrane, and connective tissue pericytes.

The hemomicrocirculatory channel of the mucous membrane of the gums of rats is extremely developed. Capillaries, as an exchange link of the microcirculatory channel, in the dynamics of the experiment characteristically responded to the introduction of food additives, primarily by thinning or thickening of the vascular wall.

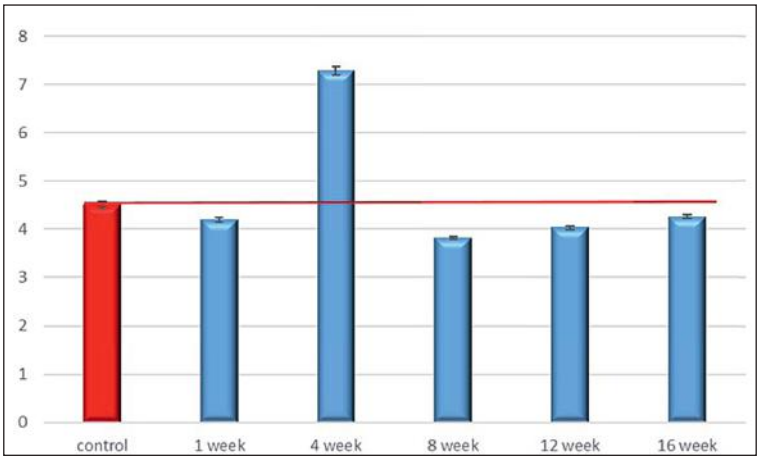

Figure 1 – Changes in the average indicators of the total diameter of the capillaries of the mucous membrane of the gums of rats in the dynamics of the experiment. A sharp increase in diameter was observed on the 4th week of the experiment and a subsequent decrease below the control level.

Thus, the average total capillary diameter for the 4th week of the experimental study increased by 38.1%, statistically significantly at $p<0.05$, compared to the control indicators. During the 8th-12th week of the experiment, the indicator of the average total diameter of the capillaries was restored to the control indicators and even became smaller and was within the limits of statistical error with the control group. At the 16th week of observation, this indicator was 4.26, which is 5.9% less compared to the control indicators, which indicates vasodilation processes **(fig. 1).**

In accordance with the changes in indicators of the average total diameter of the capillaries, there were also functional changes in the indicators of their average diameters of the lumens. Thus, after 1 week of taking the food complex, the average diameter of capillary lumens decreased by 7.7% compared to the control indicators, and already after the 4th week of the experiment, this indicator increased by 38% compared to the control group. After the 8th week of observation, this indicator again decreased by 18.1% compared to the con-

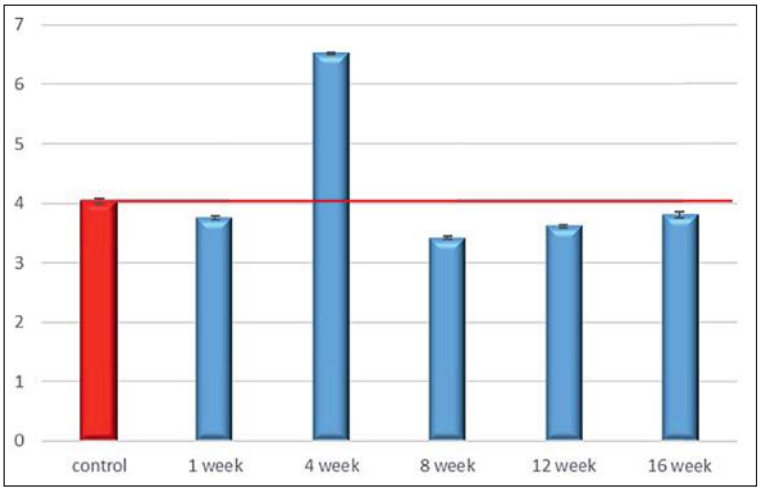

Figure 2 – Changes in the indicators of the average diameters of the lumen of the capillaries of the mucous membrane of the gums of rats in the dynamics of the experiment. Except for the 4th week, where there is a sharp increase in the diameter of the lumen, in all other cases there is a decrease in the lumen compared to the control.

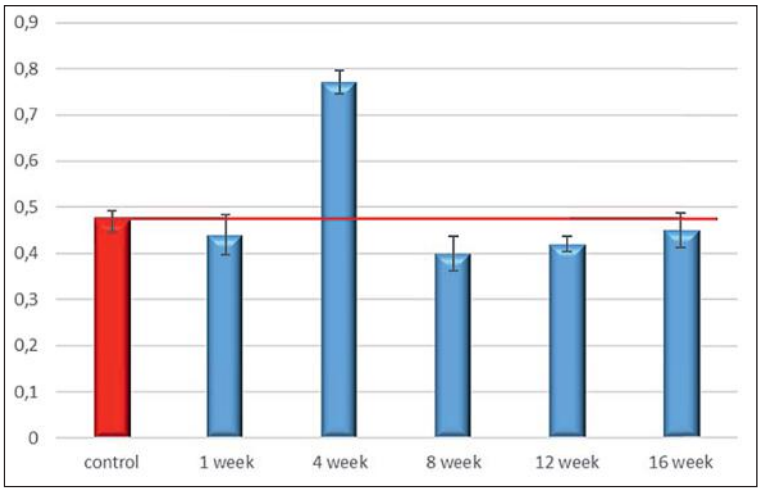

Figure 3 – Changes in the indicators of the average thickness of the vascular wall of the capillaries of the mucous membrane of the gums of rats in the dynamics of the experiment. We determine a sharp increase in the value in the 4th week. In the future, a return to approximately normal values is observed, but not a complete recovery to control values.

trol indicators. After the 12th week of the experimental study, the indicator of the average diameter of the capillary lumen increased reliably at $p<0.05$ and reached its maximum value after the 16th week of observation with a reliable increase at $p<0.05$ to 3.81, which is 5.7% less compared to control **(fig. 2)**.

A detailed analysis of the structural components of the vascular wall of capillaries showed that after 1 week of taking a complex of nutritional supplements, the vascular wall is significantly thinner at $p<0.05$ by 7% compared to control indicators. At the ultramicroscopic level, it was determined that the capillary wall underwent destructive changes. Inside the capillaries, the epithelial cells acquired a flattened appearance, and some lost their connection with the basal membrane. In the outer shell, the layer of connective tissue was significantly thinned, in comparison with the capillaries of the mucous membrane of the gums of the control group of animals. There are also destructive changes of the vascular wall in the lumen of the capillaries.

During the experimental study at week 4, a significant thickening of the capillary walls by 39% was observed compared to the control. On the 8th week of the experiment, the thickness of the walls decreased by 17.5% compared to the control, on the 12th week of the experiment, this indicator was 0.42, which is 12% less than the control indicator. During this period, at the microscopic level, restoration of the organizational structure of capillary walls due to the arrangement of epithelial cells is revealed. After the 16th week of the experimental study, there was a further significant thickening of the vascular wall at $p<0.05$, but in quantitative terms, this is all exactly 4.4% less than the indicator of the control group **(fig 3).**

After studying the results of the effect of a complex of food additives on the gum tissues of rats, we established the patterns of capillary reactions, changes in the structures of their walls, and changes occurring in the blood. According to literary sources, microvessels react to the action of factors of the external and internal environment quite differently according to the structure of the vessels themselves and the active agent. Therefore, during the morphological and morphometric study, we determined changes in the capillaries of the mucous membrane of the gums of experimental animals, as an exchange link of the hemomicrocirculatory channel [7, 8]. It is worth noting that after 1 week of the experimental study, at $p<0.05$, the average total diameter of the capillaries decreases. In addition, hyperhydration of its amorphous substance is determined on histological preparations in the connective tissue surrounding the capillary. This causes the capillaries to be clamped and the corresponding disruption of hemodynamic processes. At the same time, significant thinning of capillary walls and corresponding changes in its structural organization are noted. In our opinion, this is due to the entry into the systemic bloodstream of food additives that contain glutamate and sodium nitrite and cause both allergic reactions [9] and subsequent systemic inflammatory processes [10, 11].

During the experiment from 4 to 12 weeks, the average indicators of the total diameter, the diameter of the lumens, and the thickness of the vascular wall of the capillaries changed in response to restorative and compensatory reactions that occurred in order to neutralize the source of the alteration and restore the functions and structure of the tissues surrounding the capillaries. It is especially interesting that on the 4th week all indicators increase sharply, and on the 8th week of the experiment they all become smaller than the control. It should not be forgotten that the recovery processes did not lead to a complete restoration of normal indicators at the level of indicators of the control group of rats. This demonstrates an increase in the lumen of capillaries, a violation of the structure of the connective tissue of the mucous membrane of the gums of rats, which leads to an increase in decompensation processes and hypoxia.

After 16 weeks of observation, morphometric indicators remain at a level lower than the level of the control group of animals. At the microscopic level, numerous groups of mast cells in the stage of degranulation and in the stage of accumulation of secretory granules are visualized in the deep layers of the mucous membrane of the gums. In our opinion, mast cells play a decisive role in restoring local hemodynamics, due to the degranulation of secretory granules that contain heparin.

Conclusions.

1. Long-term intake of a complex of food additives in the body of the studied rats, containing glutamate and sodium nitrite and Ponsot 4 R, causes irreversible processes of hemodynamic disturbances of the mucous membrane of the gums with the development of hypoxic processes in the surrounding tissues.

2. Uneven thinning or thickening of the capillary wall, which we characterize as destructive changes at the ultrastructural level, leads to changes in blood properties and disruption of metabolic processes in all elements of the hemomicrocirculatory channel.

3. Restorative and compensatory reactions, which should be aimed at neutralizing the source of alteration and restoring the morphofunctional state of vessels, do not lead to a complete restoration of normal hemodynamic parameters, which in the last weeks of the study

is reflected by an increase in the average diameter of capillaries and the average diameter of the lumen of microvessels, which in turn and leads to decompensation of these processes in the mucous membrane of the gums.

4. The appearance in the deep layers of the mucous membrane of the gums of numerous groups of mast cells in the stage of degranulation and in the stage of accumulation of secretory granules indicates the cyclic processes of restoration of local hemodynamics due to the degranulation of secretory granules that contain heparin.

Our research not only confirms, but also clearly shows the effect of food additives on the body. If other studies focus their attention on the effect of a certain additive, then our experiment demonstrates what changes are characteristic of the complex effect of several at once. This is important, because most of the products we use contain a mixture of food additives. Thanks to this, our research is relevant both for theoretical workers and for practicing doctors, because it allows us to observe the chronology of changes in tissues and, accordingly, to develop treatment methods.

Prospects for further research.

The results of these experiment could be used in the next researches of food additives , especially about another types of them.

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МОРФОЛОГІЧНІ І МОРФОМЕТРИЧНІ ЗМІНИ В КАПІЛЯРАХ ГЕМОМІКРОЦИРКУЛЯТОРНОГО РУСЛА СЛИЗОВОЇ ОБОЛОНКИ ЯСЕН ПРИ ТРИВАЛІЙ ДІЇ НА НИХ ХАРЧОВИХ ДОБАВОК

Олексієнко В. В., Білаш С. М.

Резюме. Дана робота досліджує вплив комплексу харчових добавок, до яких входять глутамат натрію, нітрит натрію та Понсо 4R на ясна щурів. Дослідження спрямоване на виявлення морфологічних і гістологічних змін у стінці капілярів.

Дослідження проводилось на матеріалі з ясен щурів. Готові зрізи фарбували толуїдиновим синім та поліхромним барвником. Основними методами дослідження були морфометричний, гістологічний і статичний.

У контрольної групи ясна характеризувалися наявністю слизової оболонки без підслизової основи. Слизова оболонка зрощена із власною пластинкою, а її перший шар утворювали вузькі й високі сосочки, що ніби вростали в епітеліальний шар слизової оболонки. Сам же епітеліальний шар вистелений багатошаровим плоским незроговілим епітелієм. Досліджувані судини, тобто капіляри, визначались як структури малого діаметру округлої форми з дуже тонкою стінкою, яка складається з 3 шарів: клітин ендотелію, базальною мембраною та сполучнотканинними перицитами.

Під час дослідження були виявлені характерні зміни в трьох основних показниках: загальний діаметр, просвіт каналу та товщина стінок капілярів.

На 1 тижні визначалися незначні зменшення показників відносно контрольної групи, які різко зростали до 4 тижня дослідження. В подальшому значення зменшувалися аж до 16 тижня, але не відновлювались до показників контрольної групи. Це свідчить про недостатню дію відновних процесів у капілярах і, відповідно, про сильний вплив харчових добавок на морфологію судин при тривалій дії.

Дане дослідження показує, наскільки вплив комплексу харчових добавок потужніший за окремі добавки. Враховуючи наявність подібних добавок у більшості продуктів, цей експеримент буде цікавим практикуючим лікарям, працівникам харчової промисловості, а також іншим науковцям, що будуть вести дослідження у цьому напрямі.

Ключові слова: ясна, капіляри, епітелій, харчові добавки, морфологія.

MORPHOLOGICAL AND MORPHOMETRIC CHANGES IN CAPILLARIES OF THE HEMOMICROCIRCULATORY CHANNEL OF THE GINGIVAL MUCOSA DURING LONG-TERM EXPOSURE TO A COMPLEX OF FOOD ADDITIVES

Oleksienko V. V., Bilash S. M.

Abstract. This work investigates the effect of a complex of food additives, which include monosodium glutamate, sodium nitrite and Ponceau 4R, on the gums of rats. The study is aimed at identifying morphological and histological changes in the capillary wall.

The study was conducted on material from the gums of rats. Ready sections were stained with toluidine blue and polychrome dye. The main research methods were morphometric, histological and static.

In the control group, the gums were characterized by the presence of a mucous membrane without a submucosal base. The mucous membrane is fused with its own plate, and its first layer was formed by narrow and tall papillae, which seemed to grow into the epithelial layer of the mucous membrane. The epithelial layer itself is lined with a multi-layered flat nonkeratinized epithelium. The studied vessels, i.e. capillaries, were defined as small-diameter, rounded structures with a very thin wall, which consists of 3 layers: endothelial cells, a basal membrane, and connective tissue pericytes.

During the study, characteristic changes in three main indicators were found: the total diameter, the lumen of the channel, and the thickness of the capillary walls. In the 1st week, a slight decrease in the indicators compared to the control group was determined, which increased sharply by the 4th week of the study. Subsequently, the values decreased up to the 16th week, but did not recover to the indicators of the control group. This indicates insufficient action of regenerative processes in capillaries and, accordingly, a strong influence of food additives on the morphology of blood vessels with long-term action.

This study shows how the effect of a complex of food additives is more powerful than individual additives. Given the presence of similar additives in most products, this experiment will be of interest to practicing doctors, food industry workers, and other scientists who will conduct research in this direction.

Key words: gums, capillaries, epithelium, nutritional supplements, morphology.

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Conflict of interest:
The Authors declare no conflict of interest.

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A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

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