hemomicrocirculatory bed of the testes, which is characterized by a significant narrowing of arterioles, precapillary arterioles, hemocapillaries, significant expansion of postcapillary venules and venules , venous congestion, hypoxia, atrophic processes in the intima, apoptotic, dystrophic and necrobiotic changes of endotheliocytes, spermatogenic epitheliocytes, connective tissue components, infiltration and sclerosis.

Key words: testis, hemomicrocirculatory bed, postresection portal hypertension.

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IMMUNOHISTOCHEMICAL CHARACTERISTICS OF STRUCTURAL CHANGES IN THE CORTEX AND MEDULLA OF THE ADRENAL GLANDS UNDER THE INFLUENCE OF A FOOD ADDITIVE COMPLEX

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The importance of taste plays a significant role in everyone's life. Our taste buds play an essential role in recognizing flavors, determining the suitability of food, protecting us from harmful substances, and regulating our food intake. People always enjoy tasty food, and sweet foods contribute to positive emotions. Every day, we consume food containing a significant amount of food additives and preservatives without even thinking about their possible health consequences. Tasty but unhealthy food has become popular and affordable, and we often cannot resist the temptation to eat it. Many food additives are created to meet the needs of the food industry, as they differ from cooking at home and require products that need to be stored in a marketable condition for longer. However, these additives must be safe for consumers' health and should not mislead them. Today, there are many food additives that consumers are unaware of but which can negatively affect human organs, including the adrenal glands. Some of the most common food additives under investigation include monosodium glutamate (E 621), monosodium nitrite (E 250), and Ponceau 4R (E 124).

Consumption of a significant amount of food additives can have the following adverse effects: chest and abdominal pain, migraines, nausea, tachycardia, obesity, weakness, and allergic reactions. Particular attention should be paid to the impact of these supplements on the adrenal glands, as they can affect their function and contribute to cancer development.

Research on adrenal morphology has been ongoing for many years. In recent decades, there has been an increase in adrenal pathology as the negative impact of external and internal factors increases, leading to disorders in their structure and function. The adrenal glands play an essential role in the hypothalamic-pituitary-adrenal system and are also endocrine organs. The interest in changes in the structure of the adrenal glands is still ongoing among experimental medicine and scientists. Thanks to the improvement of computer diagnostic methods, the possibility of detecting both hormonally active and inactive benign and malignant tumors in the adrenal glands is increasing. The most common endocrine disorders are hyperaldosteronism, adrenergic and estrogen imbalance. Computed tomography significantly improves the ability to detect tumors of various origins in the adrenal glands.

Diagnosing and treating adrenal gland diseases are an urgent medical and social problem.

Key words: *monosodium glutamate, morphology, medulla, adrenal glands, sodium nitrite, Ponceau 4R, reticular zone, cortical substance, hemomicrocirculatory bed, rats, morphological and functional changes, submicroscopic changes, immunohistochemical characteristics, Ki-67.*

Connection of the publication with planned research works.

The work is a fragment of the research work of the Poltava State Medical University "Regularities of the morphogenesis of organs, tissues and vascular and nervous formations in normal, pathological and under the influence of exogenous factors", state registration № 0118U004457.

Introduction.

The value of taste plays an essential role in every person's daily life. Taste receptors regulate the recognition of food quality, protect against the ingress of harmful substances, and regulate food suitability [1-3]. People always enjoy delicious food, and sweet foods evoke positive emotions. Every day, we eat food with a huge variety of food additives and preservatives without hesitation, but even though we know about their presence, we are in no hurry to give up these foods. Tasty but unhealthy food is available in every supermarket, as it has become so widespread and widely available that it is hard to resist its pleasant smell and appearance. Many food additives have emerged in response to the need to mass produce and store food products [4].

Food additives are needed to maintain the attractive appearance and long-term food storage. More than a thousand existing food additives can be found on supermarket shelves without the consumer even knowing about their existence [5-6]. Not all manufacturers list additional harmful food additives in their products. Each food additive affects human organs to a certain extent and in a certain amount, in our case, the adrenal glands, which are vital organs of the endocrine system, involved not only in the neurohumoral regulation of water-salt, carbohydrate protein and fat metabolism, affect the function of other endocrine glands, participate in protective and adaptive reactions, are a source of stem cells and act as the main link in the stress-response system of the body as a whole [7]. Monosodium glutamate (E621), monosodium nitrite (E250), and Ponceau 4R (E124) are among the most common in the food industry, and we are studying their effects on the adrenal glands [8-9].

The aim of the study.

To determine the expression of Ki-67 in the cortex and medulla of the adrenal glands of rats after exposure to a complex of food additives (sodium glutamate, sodium nitrite and Ponceau 4R).

Object and research methods.

The study was conducted on white outbred rats weighing 0.350±0.15 kg, kept in the vivarium of Poltava State Medical University. All experimental studies were carried out in compliance with the requirements of 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 Research and Other Scientific Purposes (Strasbourg, 1986).

The control group of rats used oral drinking water and physiological saline. Rats from the experimental group were orally injected with a 10% solution of sodium nitrite (E250); monosodium glutamate (E621) was administered 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. Dosages of food additives were two times lower than the permissible norm in food products. Rats were removed from the experiment after 1, 4, 8, 12 and 16 weeks by using ether anesthesia followed by euthanasia.

For histological examination, pieces of adrenal glands were fixed for 6-8 hours in 10% neutral buffered formalin (Biognost, Croatia). Tissue processing was performed in a LOGOSone histoprocessor (Milestone, Italy). Paraffin sections of adrenal glands (5 μm thick) were prepared on an AMR 400 manual rotary microtome (Amos science, Australia), stained with hematoxylin and eosin (Biognost, Croatia) and evaluated using a Nikon Eclipse Ci light microscope (Nikon, Japan) with photodocumentation using Sigeta M3CMOS 14000 digital camera.

Immunohistochemical studies were performed on thin formalin-paraffin sections of rat adrenal glands. Recombinant rabbit monoclonal primary antibodies Anti-Ki-67 (Cat. No. ab16667, Abcam, USA) were used to determine cell proliferation. Antibody detection was performed using the Mouse/Rabbit PolyVue™ HRP/DAB polymer system (Diagnostic BioSystems, USA). Sections were stained with hematoxylin M (Biognost, Croatia).

The results of immunohistochemical reactions were evaluated by counting the number of positively stained areas. Ki-67 intensity was assessed by immunohistochemical reaction using a semi-quantitative method that considered the absence, presence and intensity of staining cells with the biomarker. The number of labeled cells with Ki-67 was determined as a percentage, the percentage of cells with the biomarker was counted in ten fields of 100 cells, the average percentage was determined and its value was interpreted as the labeling index (LI%). The investigated Ki-67 protein was evaluated in cells of the cortical (glomerular, fasciculate, reticular zone) and medulla of rat adrenal glands. When evaluating the expression of Ki-67, a positive reaction was considered as such in the cortex and medulla of the adrenal glands of rats. When evaluating immunohistochemical staining, a positive reaction with Ki-67 was represented by brown staining of cell nuclei of varying intensity. The intensity of expression was assessed by a semi-quantitative method based on the severity and integrity of the color of pathological changes. Rats that did not use food supplements were used as controls.

Determining the level of expression of the nuclear proliferation protein Ki-67 makes it possible to assess the proliferative activity of cells of the adrenal glands of rats after the use of a complex of food additives.

Research results and their discussion.

The Ki-67 antigen is the most well-known proliferation factor. The role of this antigen in the vital activity of cells remains unknown, but since it is synthesized in almost all phases of the mitotic cycle, except for the resting phase (G0) and reflects the indicator of the general prolif-

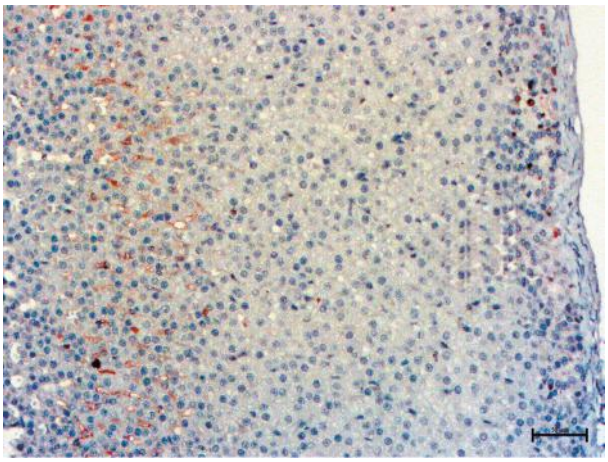

Figure 1 – Structural organization of rat's adrenal gland with Ki-67-positive cells (colored brown). Mayer's hematoxylin staining. Magnification: okh. 10, obh. 10.

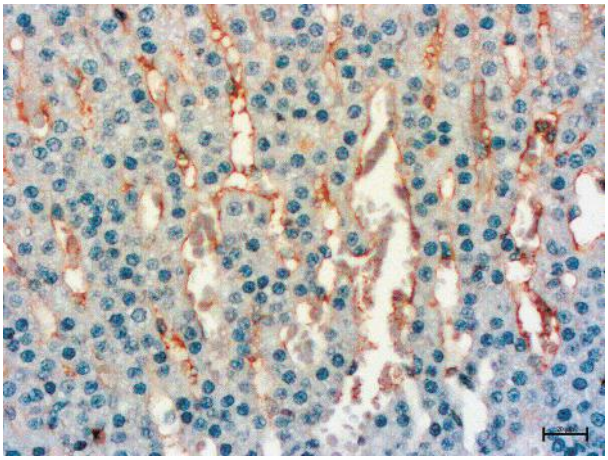

Figure 2 – Ki-67 positive cells in the wall of fenestrated cortical sinusoidal capillaries. Staining with Mayer's hematoxylin. Magnification: okh. 10, obh. 40.

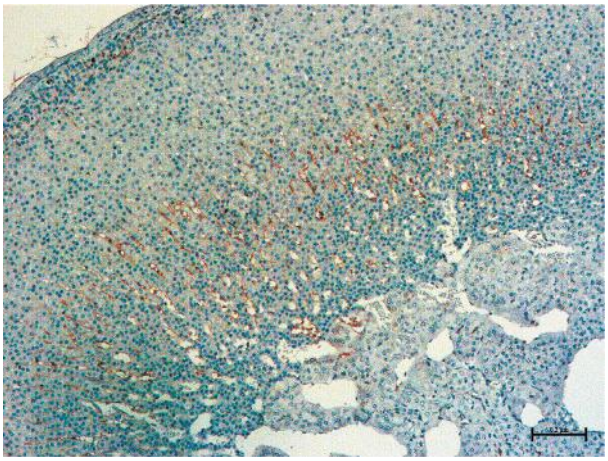

Figure 3 – Structural organization of cortex and medulla of rats with Ki-67 positive cells (colored brown). Mayer's hematoxylin staining. Magnification: okh. 10, obh. 10.

erative pool, this protein is used as a universal marker of cell proliferation [10].

During the morphometric study, we established that the hemomicrocirculatory bed of the adrenal glands of rats was a well-developed multi-component structure. After experimental modeling of the effect of a complex of food additives on the adrenal glands of rats and the study of the expression of Ki-67, we discovered the regular reactions of the vessels of the cortex and medulla. After all, the hemomicrocirculatory bed's reaction to the external and internal environment factors is quite specific. Changes were analyzed depending on the location of vessels: in the cortex (glomerular, fasciculate, reticular zone) and medulla [11].

A clear section of the arteriole contour is observed at the border of the glomerular and fasciculate zones without signs of hyperhydration.

Ki-67 positive endotheliocytes were visualized in the walls of arterioles of the reticular zone of the adrenal glands of rats in the first week of the experimental study. The section of the arteriole itself remained of the correct oval shape, and the surrounding structural components were without signs of hyperhydration [12-13].

During a detailed analysis of microphotographs, it was established that Ki-67-positive cells were localized at the border of the adrenal gland capsule and cortex from 1 to 5 cells in the field of view. They were locally determined in large numbers and formed clusters of 2 to 10 in the field of vision **(fig. 1)**.

By the method of immunohistochemistry in the fasciculate and reticular zones of the cortical substance, Ki-67 positive cells were determined most often in the wall of fenestrated cortical sinusoidal capillaries that supply blood to the cortical substance and then pass into the fenestrated sinusoidal capillaries of the medulla, and form strands **(fig. 2)**. Erythrocytes are well visualized in the capillary lumens.

The nuclei of epitheliocytes were uniformly stained, and the proliferation marker Ki-67 expression could be observed in their nuclei. Single Ki-67 positive cells were hardly detected in the fascicular zone of the adrenal glands. They could be found 1-2 in the field of vision. In the fasciculate zone of the cortical substance of the adrenal glands, Ki-67 positive cells were single and located at a considerable distance from each other.

In rats of the control group, the content of Ki-67 in the capsule of the adrenal glands was 3.4%, in the glomerular zone – 6%, in the fasciculate zone, the content of Ki-67 was 12%, in the reticular zone – 9%, in the peripheral zone of the medulla – 3%, in the central zone of the brain substance – 12% **(fig. 3)**.

After modeling the experimental effect of a complex of food additives on the adrenal glands of rats, we discovered regular changes in the structural components of the cortex and medulla. The content of Ki-67 was significantly higher after using a complex of food additives compared with the control values.

In the first week of the experimental study, Ki-67 expression indicators were: capsule – 1.8%, which is 47.05% less than the control value; glomerular zone 12.6% – high proliferative activity, which is 110% more than control values; fasciculate zone 17%, which is 41.6% higher than the control value; reticular zone – 18%, which is 100% higher than the initial control values; the peripheral zone of the medulla – 2%, which is 33.3% less than the control value; of the central zone of the medulla – 8%, which is 33.33% less than the control value.

After the fourth week of the experimental study, the expression index of Ki-67 in the capsule of the adrenal glands decreased slightly and amounted to 2.1%, which is 38.23% lower than the control value. In the glomerular zone, the Ki-67 expression index increased to 8.2%, 36.67% more than the control values. In the fasciculate

zone of the adrenal glands of rats, the expression index of Ki-67 decreased slightly and amounted to 11.6%, which is 3.33% less than the control indicators. The proliferation index of the reticular zone was 6.4%, which is 28.89% lower than the control values. The peripheral zone of the medulla had Ki-67 expression values 170% higher than the control values, equal to 8.1%. The indicators of the central zone in the fourth week showed an expression rate of Ki-67 of 10%, 16.69% less than the control indicators.

In the eighth week of the experimental study, the Ki-67 expression index in the capsule of the adrenal glands was 1.9%, 44.11% lower than the control values. In the glomerular zone of the cortex of the adrenal glands of rats in the eighth week, the rate of Ki-67 expression was 9.2%, 53.33% higher than the control. Fasciculate and reticular zones had insignificant deviations from the control values – 10.1% and 9.8%, less than 15.83% and more by 8.89%, respectively. Peripheral and central zones of the medulla of rat adrenal glands showed the following percentage of Ki-67 expression: peripheral zone – 13.2%; the central zone – 14.3%, these indicators are higher than the control values by 340% and 19.16%, respectively.

At week 12, the Ki-67 expression index in the capsule of the adrenal glands did not change significantly compared to the first and second weeks and was 1.4%, which is 58.82% less than the control value. Ki-67 expression indicators in the glomerular, fasciculate, and reticular zones of the cortical substance of the adrenal glands corresponded to the following indicators: glomerular zone – 8.1%; fasciculate zone-14.3%; reticular zone 8.5%. These indicators were higher than the control values by 35%, by 19.16%, only in the reticular zone they were lower by 5.88%.

In the 16th week of the experimental study, the expression of Ki-67 in the capsule of the adrenal glands increased to 2.6% compared to the previous weeks, but it was 23.52% lower than the control value. Indicators of expression of Ki-67 in the cortical substance did not differ significantly in the last weeks of the study: glomerular zone – 8.3% – 38.33% more than the control; reticular zone – 9.1% – 1.11% more than the control; the expression rate increased significantly only in the fasciculate zone of the cortical substance and amounted to 17.2% – 43.33% higher than the control value.

Conclusions.

The data obtained during the experiment allow us to conclude that the influence of the complex of food additives sodium glutamate, sodium nitrite and Ponceau 4R is a mechanism of activation of the focus of inflammation and changes in the structural elements of the adrenal glands and hemomicrocirculatory bed.

According to the data of an immunohistochemical study, it was established that in the cortex and medulla of the adrenal glands, Ki-67 positive cells are elements of the local protective barrier. The largest number of Ki-67-positive cells was determined in the walls of fenestrated capillaries that permeate the cortex and medulla of the adrenal glands. Ki-67-positive cells formed clusters of 2-10 cells at the border of the glomerular and reticular zones.

The total number of Ki-67-positive cells varied according to the week of the experimental study. Starting from the first week, the number of cells expressing Ki-67 increases dramatically in the adrenal cortex's glomerular, fasciculate and reticular zones. In the fourth week of the experiment, the level of labeled cells with Ki-67 reached almost control values. However, at the 12th week, the number of cells expressing Ki-67 increases in the cortex and the medulla. On the 16th week, the indicators remain within the 12th week, with an increase in the number of Ki-67-positive cells in the fasciculate zone of the cortical substance of the adrenal glands.

Prospects for further research.

Further immunohistochemical study of markers of inflammation and proliferation is planned.

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ІМУНОГІСТОХІМІЧНА ХАРАКТЕРИСТИКА СТРУКТУРНОЇ ПЕРЕБУДОВИ КІРКОВОЇ І МОЗКОВОЇ РЕЧОВИНИ НАДНИРНИКІВ ЗА УМОВ ДІЇ ХІМІЧНИХ ХАРЧОВИХ ДОБАВОК У КОМПЛЕКСІ

Донченко С. В., Білаш С. М.

Резюме. Дана робота містить визначення експресії Ki-67 у кірковій та мозковій речовині надниркових залоз щурів після впливу комплексу харчових добавок (глутамат натрію, нітрит натрію та Понсо 4R).

Ki-67 є білком, який є маркером клітинної проліферації або ділення клітин у тканинах організму. Виразність Ki-67 у клітинах може свідчити про те, наскільки активно вони діляться і ростуть. Результати цих досліджень можуть допомогти в лікарській практиці, особливо в онкології, для оцінки пухлин та визначення стратегії лікування, оскільки пухлини з високою виразністю Ki-67 часто ростуть швидше і більш агресивно.

За результатами імуногістохімічного дослідження було встановлено, що Ki-67-позитивні клітини, згідно з результатами дослідження, виконують функцію складових локального оборонного бар'єру в кірковій та мозковій речовинах надниркових залоз. Найвища кількість Ki-67-позитивних клітин виявлена в стінках фенестрованих капілярів, які пронизують кіркову та мозкову речовину надниркових залоз. На межі клубочкової та сітчастої зон виявлені групи клітин, які виявили позитивність до Ki-67, кількість яких становила від 2 до 10 клітин.

Загальна кількість Ki-67-позитивних клітин змінювалася протягом тижня експериментального дослідження. Вже з першого тижня спостерігалося різке зростання кількості клітин, що експресують Ki-67, в клубочковій, пучковій та сітчастій зонах кіркової речовини надниркових залоз. На четвертому тижні експерименту рівень позитивних на Ki-67 клітин майже досяг контрольних значень. Однак на 12-му тижні кількість клітин, що експресують Ki-67, різко збільшилася як в кірковій, так і в мозковій речовині. На 16-му тижні показники залишилися на рівні 12-го тижня, з подальшим зростанням кількості Ki-67-позитивних клітин в пучковій зоні кіркової речовини надниркових залоз.

Ключові слова: глутамат натрію, морфологія, мозкова речовина, надниркові залози, нітрит натрію, Понсо 4R, сітчаста зона, кіркова речовина, гемомікроциркуляторне русло, щури, морфофункціональні зміни, субмікроскопічні зміни, ендокриноцити, Ki-67.

IMMUNOHISTOCHEMICAL CHARACTERISTICS OF STRUCTURAL CHANGES IN THE CORTEX AND MEDULLA OF THE ADRENAL GLANDS UNDER THE INFLUENCE OF A FOOD ADDITIVE COMPLEX

Donchenko S. V., Bilash S. M.

Abstract. This work contains the determination of Ki-67 expression in the cortex and medulla of the adrenal glands of rats after exposure to a complex of food additives (sodium glutamate, sodium nitrite, and Ponceau 4R).

Ki-67 is a protein marker of cell proliferation or cell division in body tissues. The expression of Ki-67 in cells can indicate how actively they are dividing and growing. The results of these studies can help in medical practice, especially in oncology, to evaluate tumors and determine treatment strategies since tumors with high expression of Ki-67 often grow faster and more aggressively.

According to the results of an immunohistochemical study, it was established that Ki-67 positive cells perform the function of components of the local defense barrier in the cortical and medullary substances of the adrenal glands. The highest number of Ki-67-positive cells was found in the walls of fenestrated capillaries that penetrate the cortex and medulla of the adrenal glands. On the border of the glomerular and reticular zones, groups of cells showed positivity to Ki-67, the number of which was from 2 to 10 cells.

The total number of Ki-67-positive cells varied during the week of the experimental study. Already from the first week, a sharp increase in the number of cells expressing Ki-67 was observed in the glomerular, fasciculate and reticular zones of the cortical substance of the adrenal glands. In the fourth week of the experiment, the level of Ki-67 positive cells almost reached the control values. However, at week 12, the number of cells expressing Ki-67 increased dramatically in both the cortex and medulla. On the 16th week, the indicators remained at the level of the 12th week, with a further increase in the number of Ki-67-positive cells in the fasciculate zone of the cortical substance of the adrenal glands.

Key words: monosodium glutamate, morphology, medulla, adrenal glands, sodium nitrite, Ponceau 4R, reticular zone, cortical substance, hemomicrocirculatory bed, rats, morphological and functional changes, submicroscopic changes, immunohistochemical characteristics, Ki-67.

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