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THE IMPACT OF FOOD ADDITIVES COMPLEX ON THE STRUCTURAL ORGANIZATION OF PULMONARY DIFFUSE LYMPHOID TISSUE SHOWN IN THE EXPERIMENT

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The paper presents the findings of morphometric and morphological studies of the diffuse lymphoid tissue of rat lungs under the combined effect of the food additives. It has been found that the condition of the diffuse lymphoid tissue of rat lungs, under the impact of the complex of food additives comprise of monosodium glutamate, sodium nitrite and Ponceau 4R, reflects the reaction of elements of the local defense barrier to the action of the components of the food additives' complex, primarily functioning as antigens. This is manifested by the activation of processes of antigen-dependent differentiation of cells of the lymphoid lineage, undulating changes in the morphometric parameters and the degree of vascularization of the diffuse lung tissue.

Key words: food additives, diffuse lymphoid tissue, capillaries, lymphocytes, plasma cells, macrophages, lungs, rats.

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ВЖИВАННЯ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК ВПЛИВАЄ НА СТРУКТУРНУ ОРГАНІЗАЦІЮ ДИФУЗНОЇ ЛІМФОЇДНОЇ ТКАНИНИ ЛЕГЕНЬ В ЕКСПЕРИМЕНТІ

В роботі представлені результати морфометричного та морфологічного дослідження дифузної лімфоїдної тканини легень щурів при комплексній дії харчових добавок. Встановлено, що стан дифузної лімфоїдної тканини легень щурів під дією комплексу харчових добавок глутамату натрію, нітриту натрію та Понсо 4R, відображає реакцію елементів місцевого захисного бар'єру, на дію складових комплексу, які перш за все виступають у ролі антигенів, що відображається активацією процесів антигензалежного диференціювання клітин лімфоїдного ряду, змінами з хвилеподібним характером морфометричних показників та ступеня васкуляризації дифузної легеневої тканини.

Ключові слова: харчові добавки, дифузна лімфоїдна тканина, капіляри, лімфоцити, плазмоцити, макрофаги, легені, щури.

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Diffuse lymphoid tissue is localized in the rat lung parenchyma, represented by the aggregations and unorganized lymphocytic clusters surrounded by the connective tissue membrane in the interalveolar interstitial tissue and may be at different stages of differentiation. The main function of diffuse lymphoid tissue is to capture antigens that enter the lungs and to generate local immunity to various antigens [14].

It is known that toxins and inflammatory mediators primarily enter the pulmonary capillaries, making them among the first to be affected. This leads to disturbances in the microcirculatory system and damage to the organ's parenchyma, with the development of interstitial and alveolar edema. The damage to the respiratory part of the lungs is attributed to endothelial-epithelial dysfunction [10].

The widespread use of food additives in food products [5] is a matter of particular concern for both domestic and foreign researchers, and no data on their combined effect have been found [3].

The analysis of the content of food additives in products from both foreign and domestic production has found that the most prevalent additives are monosodium glutamate, sodium nitrite and Ponceau 4R.

Preclinical studies have associated the consumption of monosodium glutamate with low-grade inflammation, metabolic disorders and precancerous lesions. Furthermore, there have been reports of a relationship between monosodium glutamate consumption and various alterations in lymphocytes and thymocytes [15].

Based on the findings on the dynamics of changes in markers of bioenergetic processes and cytolysis in rats after sodium nitrite consumption, the poisoning of rats from different age groups is accompanied by the development of membrane-destructive processes in the body, resulted in suppression of tissue respiration processes, including the activity of mitochondrial enzymes such as succinate dehydrogenase and cytochrome oxidase, leading to hypoxia [2].

In the food industry, food colorants are widely used to provide products with the necessary color or shade. When the colorants enter the body as haptens and bind to proteins such as serum albumin and others, they become full-fledged antigens to which antibodies are synthesized. The allowed sanitary and hygienic standards are usually exceeded, which enhances their allergenicity [7, 8, 11].

Experimental modeling in animals is one of the main methods for studying the regularities of the development of pathological processes that often occur in clinical practice. For the objective comparative assessment of experimental data and their subsequent extrapolation to humans, it is important to know the basic morphometric parameters of organs and tissues [1].

The purpose of the study was to establish the dynamics of morphometric parameters of diffuse lymphoid tissue and the number of exchange vessels in the rat lungs under normal conditions and in the combined effect of food additives, namely, monosodium glutamate, sodium nitrite and Ponceau 4R.

Materials and methods. 84 mature male rats were involved into the study. The rats of control group consumed drinking water and were administered with saline per os. The rats of the experimental group, with access to water ad libitum, were administered with 0.6 mg/kg sodium nitrite, 20 mg / kg monosodium glutamate and 5 mg / kg Ponceau 4R in 0.5 ml of distilled water once daily per os. The doses of food additives were twice lower the allowable normal rate in food products. The "open field" test was used to evaluate the rats' adaptive behavior [13]. The animals were sacrificed within 1, 4, 8, 12 and 16 weeks under thiopentone anesthesia overdose. After animals' euthanasia, the fragments of the lungs were fixed in 10 % formalin solution. Subsequently, the pieces of the lungs were embedded into paraffin, using the conventional technique [9]. Sections of 5-10 μm thick were obtained using the ARM 3600 microtome. After staining with hematoxylin and eosin, the sections were placed in polystyrene and studied under the light microscope. The digital microscope, equipped with the Levenhuk D740T digital microphoto attachment, and adapted software have been used for microphotography and morphometric study. Statistical processing of morphometric data was performed using the Excel program [4, 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 №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. Morphometric study of the rat pulmonary diffuse lymphoid tissue showed that the average outer diameter of lymphoid structures was 157.74±6.29 μm, the average thickness of the membrane was 28.65±1.52 μm, and the calculation of the average number of capillaries in the field of view was 38.41±0.07 (Table 1).

Table 1

Rat pulmonary diffuse lymphoid tissue

Parameter	Outer diameter	Thickness of the membrane	Number of capillaries
Control group	157.74±6.29	28.65±1.52	38.41±0.07
Week 1	179.57±17.83 *	24.55±1.20 *	38.22±0.09 *
Week 4	276.22±10.37 *,**	23.08±1.92 *	32.51±0.08 *,**
Week 8	131.22±0.83 *,**	10.58±0.11 *,**	52.40±0.10 *,**
Week 12	125.72±0.13 *,**	6.58±0.25 *,**	47.42±0.22 *,**
Week 16	187.86±0.45 *,**	11.77±0.34 *,**	29.41±0.16 *,**

Note * – P<0.05 compared to the control group; ** - P<0.05 compared to the previous time period of the observation.

Histological study of rats' lung specimens revealed that the diffuse lymphoid tissue was localized among the alveolar apparatus and predominantly had a rounded shape. Its structure was composed of reticular cells, externally covered with connective tissue membrane. The cytological composition was mainly represented by lymphocytes, distributed diffusely, and scarce macrophages. A large number of capillaries were visualized within the diffuse lymphoid tissue (Fig. 1).

Following one week of consumption of the complex of food additives, the mean values of the outer diameter significantly increased by 13.84%, accounting for 179.57±17.83 μm. The parameters of

membrane's thickness were significantly lower by 14.31% compared to the control values, accounting for 24.55±1.20 μm ($p<0.05$). The average number of capillaries significantly decreased by 0.49%, compared to the control group, accounting for 38.22±0.09 ($p<0.05$).

Consumption of the complex of monosodium glutamate, sodium nitrite and Ponceau 4R on week 4 of the experiment led to a significant increase in the outer diameter of diffuse lymphoid tissue clusters in the lungs of rats compared to the previous time period of the experiment by 53.82%, accounting for 276.22±10.37 μm. This value was also significantly greater by 75.11% compared to the parameters of the control group ($p<0.05$). The average thickness of the membrane was 23.08±1.92 μm, which did not significantly differ from the values of week 1 of the experiment but was significantly lower by 19.44% compared to the parameters of the control group ($p<0.05$). The average number of capillaries was 32.51±0.08, which was significantly lower by 14.94% compared to the values on week 1 of the experiment and also significantly lower by 15.36% compared to the parameters in the control group of animals ($p<0.05$).

Histological study revealed reorganization of the cellular composition in the elements of diffuse lymphoid tissue, manifested by a decrease in the number of lymphocytes and the appearance of a large number of plasma cells and macrophages. Capillaries were visualized much worse and were in a spasmodic state (Fig. 2).

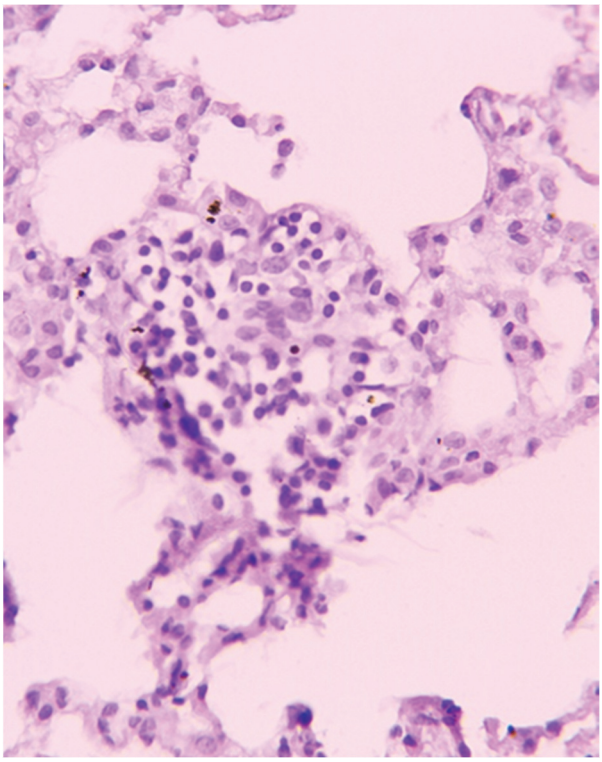

Fig. 1. The diffuse lymphoid tissue in the parenchyma of the lungs of the control group of rats. H&E stain. Oc. lens: 10×magnification; ob. lens: 40× magnification.

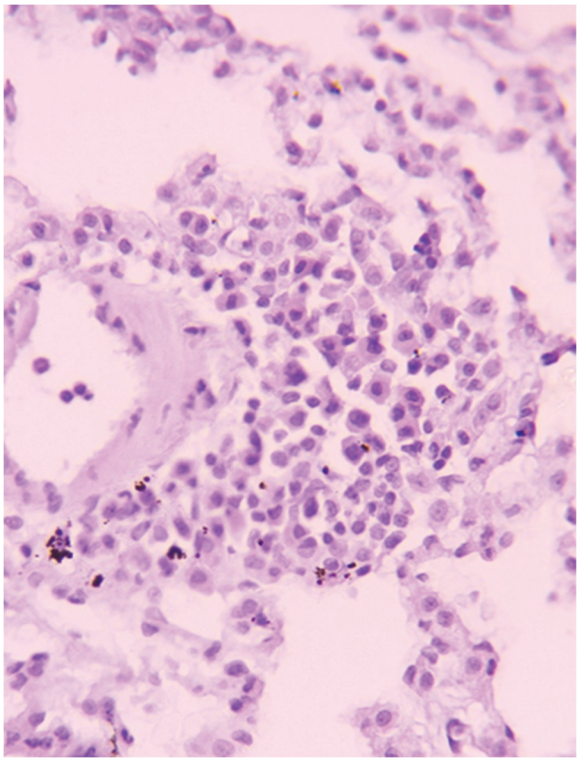

Fig. 2. Reorganization of the cellular composition of diffuse lymphoid tissue of rat lungs on week 4 of consuming the complex of food additives. H&E stain. Oc. lens: 10×magnification; ob. lens: 40× magnification.

The complex action of food additives on week 8 resulted in significant decrease of the mean values of the outer diameter by 52.49% compared to the previous time period of the experiment, accounting for 131.22±0.83 μm, which was also by 16.81% significantly lower than the values in the control group ($p<0.05$). The average thickness of the membrane was by 54.16% significantly lower than the mean morphometric values on week 4 of the experiment, accounting for 10.58±0.11 μm, and was by 63.07% significantly lower than the value in the control group of animals ($p<0.05$). The average number of capillaries significantly increased by 61.18% compared to the previous time period of the experiment, accounting for 52.40±0.10 FOV, which was by 36.42% significantly lower than the control values ($p<0.05$).

The diffuse lymphoid tissue in histological specimens appeared as small, irregularly shaped formations where the interstices between reticular cells contained a small number of lymphocytes with scarce macrophages. The majority of macrophages were localized within the alveolar lumen (Fig. 3).

By week 12 of the experiment, consumption of the complex of food additives led to decrease in the average outer diameter of the diffuse lymphoid tissue by 4.19% compared to the values of week 8 of the experiment, accounting for 125.72±0.13 μm, and also by 20.30% significantly lower than the values in the control group of animals ($p<0.05$). The average thickness of the membrane of lymphoid tissue clusters decreased by 37.81% compared to the previous term of the experiment and accounted for 6.58±0.25 μm, which was by 77.03% significantly lower than the values in the control group of rats ($p<0.05$). The number of capillaries significantly decreased by 9.50% and was 47.42±0.22 FOV, which was by 23.46% significantly greater than the values in the control group of animals ($p<0.05$).

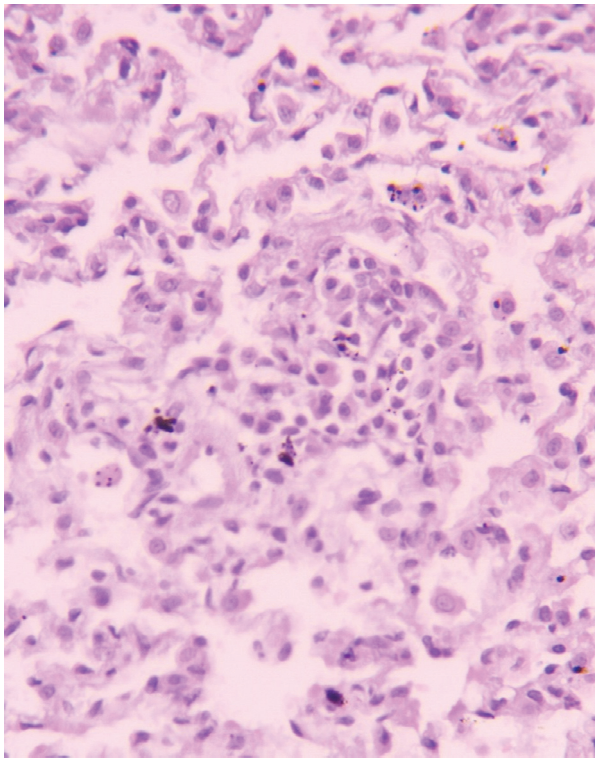

Fig. 3. The diffuse lymphoid tissue of rat lungs on week 8 of consumption of the complex of food additives. H&E stain. Oc. lens: 10×magnification; ob. lens: 40× magnification.

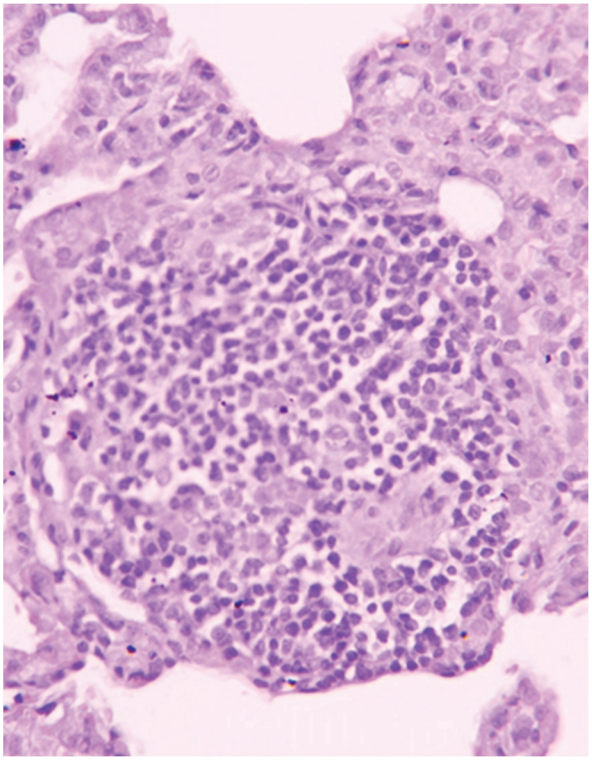

Fig. 4. Antigen-dependent differentiation of cells of the lymphoid lineage on week 16 of consumption of the complex of food additives. H&E stain. Oc. lens: 10×magnification; ob. lens: 40× magnification.

On week 16 of the experiment, the consumed complex of food additives, comprising of monosodium glutamate, sodium nitrite, and Ponceau 4R, led to a significant increase in the average outer diameter of the diffuse lymphoid tissue of rat lungs by 49.43%, compared to the previous period of the study, and accounted for 187.86±0.45 μm, which was also by 19.09% significantly greater than the values in the control group ($p<0.05$). The average thickness of the membrane significantly increased by 78.76% compared to the values on week 12, accounting for 11.77±0.34 μm, though was by 58.92% significantly lower than the mean values in the control group ($p<0.05$). The average number of capillaries significantly decreased by 37.98% compared to the previous term of the study, which was also by 23.43% significantly lower than their number in the control group of animals, accounting for 29.41±0.16 FOV ($p<0.05$).

Microscopic study revealed the process of antigen-dependent differentiation of cells of the lymphoid lineage, with a predominance of plasma cells grouped among lymphocytes and reticular cells (Fig. 4).

Thus, the diffuse lymphoid tissue was arranged in the form of orbicular clusters in the interalveolar apparatus of the rat lungs, formed by the cells of the lymphoid lineage localized between reticular cells and covered externally by a connective tissue membrane.

Consumption of the complex of food additives comprised of monosodium glutamate, sodium nitrite and Ponceau 4R showed the undulating changes in the morphometric parameters of their linear size throughout the experiment. This was expressed in a significant increase in their mean values by 75.11% on week 4 of the experiment, followed by a 20.30% decrease on week 12, and subsequent increase by 19.09% compared to the control group of animals ($p<0.05$). Apparently, the parameters reflected changes in the morphofunctional state of the diffuse lymphoid tissue of the rat lung, which were traced during the antigen-dependent differentiation of lymphoid cell populations in response to the action of the components of the

complex of monosodium glutamate, sodium nitrite and Ponceau 4R. This was subsequently reflected in the phenomenon of nonspecific inflammation and confirmed by the findings of the previously conducted studies on the impact of components of the food additives on the state of bronchus-associated lymphoid tissue [14], as well as the effects of other etiological factors on organs and tissues [9]. The undulating changes in the morphometric parameters of the linear size of the diffuse lymphoid tissue was reflected in histological specimens, where a tendency to change in the number of lymphocytes and plasma cells was observed. This change was caused by the action of a nonspecific factor acting as antigens [12] on lung tissue, leading to cell damage and the subsequent formation of an immune response. Throughout the experiment, undulating changes in the vascularization of the diffuse lymphoid tissue were noted in response to the action of the complex of food additives, reflecting the process of forming an immune response to a nonspecific alternative factor.

Conclusion

The condition of the diffuse lymphoid tissue of the rat lungs under the effect of the complex of food additives, comprising of monosodium glutamate, sodium nitrite and Ponceau 4R, reflects the response of local defense barrier elements to the action of the components of the food additive complex. These components primarily act as antigens, which is manifested in the activation of antigen-dependent differentiation processes of lymphoid cell populations. This is evidenced by undulating changes in morphometric parameters and the degree of vascularization of the diffuse pulmonary tissue.

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