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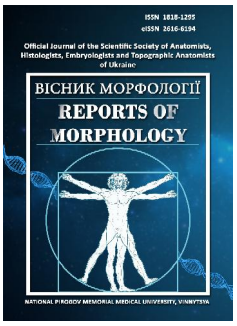

Changes in the structural organization of lymph nodes during short-term exposure to monosodium glutamate

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Monosodium glutamate is a common food additive that belongs to the group of flavor enhancers used in a wide range of food products. The potentially negative impact of monosodium glutamate on human health prompts us to question the safety of its widespread use. The article presents and analyzes the data of an experimental study conducted on 40 white male and female rats of reproductive age (2.5-3.5 months old) weighing 120-190 g. The purpose of the study is to study histological, morphometric, and ultrastructural changes in the lymph nodes of rats under conditions of short-term exposure to monosodium glutamate. An experimental group of animals (10 male rats, 10 female rats), which was on a standard vivarium diet, was given monosodium glutamate at a dose of 0.07 g/kg of rat body weight by pipette every day at the same time for four weeks, followed by free access to water and food. In animals of the intact group, the structure of mesenteric lymph nodes corresponded to the species norm. In the experimental group of animals, after four weeks of monosodium glutamate exposure, it was found that the paracortical area contained a g larger number of post-capillary venules with a high endothelium compared to the intact and control groups of animals. Changes in the vascular bed were established, in particular, the arteries both in the thickness of the organ and in its hilum contain a thickened wall, their lumen is full of blood. Veins are also full-blooded, dilated and deformed. Swelling of the parenchyma of nodes and signs of immune activity are observed. There is a significant decrease in the relative area of lymphoid nodules and the mantle zone, an increase in the relative area of the medullary substance of the node, corticomedullary index, germinal centers, paracortical area, and medullary cords. Part of lymphocytes of all populations with signs of apoptosis. The other part has an uneven karyolemma contour, the nucleolus is not visualized in all lymphocytes, the cytoplasm is clear and contains organelles. Mitochondria are hypertrophied with a light matrix. Therefore, even a short-term daily exposure to monosodium glutamate, namely four weeks, causes changes in the structural organization of mesenteric lymph nodes.

Keywords: *experiment, monosodium glutamate, lymphatic node, cortical substance, medullary substance, lymphocytes.*

Introduction

Monosodium glutamate (MSG) is a monosodium salt of glutamic acid, which is often found in nature and exists as a free substance glutamate, and as bound to other amino acids in protein [2, 6, 12]. It is a common food additive, belongs to the group of flavor enhancers used in a wide range of food industry products, for example, soups, sauces, puddings, chips, meat products and mixed seasonings [3, 11]. In addition to enhancing the taste of foods, MSG increases appetite, which causes an increase in the amount of food consumed, leading to a high-calorie diet [1]. Long-term use of monosodium glutamate causes a number of diseases and complications that are difficult to treat [14].

The potentially negative impact of monosodium glutamate on human health prompts us to question the safety of its widespread use [12]. It is known that monosodium glutamate has a negative effect on the structures of the central nervous system, causes insulin resistance, diabetes, hypertension, metabolic syndrome, etc. [17, 19]. Its listed negative effects are still

underestimated, and at the same time, people continue to consume increasing amounts of monosodium glutamate, not knowing about the possible consequences.

Therefore, studying the influence of additives, in particular monosodium glutamate, on the state of the body as a whole and the structure of tissues and organs directly is an urgent issue today, both medically and socially [14].

Lymphoid organs protect the body against exposure to various exo- and endoantigens. One of them are lymph nodes, which are also called "biological filters" [7, 9, 12, 13]. By "filtering" lymph through themselves, they are the first to react to antigens "settled" in them [8, 10]. That is why the functional capacity of these organs is undeniably extremely important for the stability and condition of the entire organism.

The purpose of the study: to study the histological, morphometric and ultrastructural changes of the lymph nodes of rats under conditions of short-term exposure to monosodium glutamate.

Materials and methods

The study was conducted on 40 white male and female rats of reproductive age (2.5-3.5 months old) weighing 120-190 g.

The structure of mesenteric lymph nodes under physiological conditions was studied in 10 animals of the intact group (5 male rats, 5 female rats). An experimental group of animals (10 male rats, 10 female rats), which was on a standard vivarium diet, was given monosodium glutamate at a dose of 0.07 g/kg rat body weight with a pipette every day at the same time for four weeks, with subsequent free access to water and food. A control group of animals (5 male rats, 5 female rats) fed a standard vivarium diet received saline solution (0.9 % NaCl solution) daily with a pipette for four weeks.

All experimental animals were kept in the vivarium of Danylo Halytsky Lviv National Medical University. The research was conducted in accordance with the provisions of the "European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), Council of Europe Directives 86/609/EEC (1986), Law of Ukraine No. 3447-IV "On the Protection of Animals from cruelty", general ethical principles of experiments on animals, adopted by the First National Congress of Ukraine on Bioethics (2001).

Preparation of preparations of mesenteric lymph nodes for histological and electron microscopic examination was carried out according to generally accepted methods. Spleen pieces were fixed with a 1.5 % solution of osmium tetroxide in a 0.2 M solution of sodium cacodylate at pH 7.2 for 2-2.5 hours in the cold. Sections were made on an ultramicrotome UMTP-6M with a diamond knife (DIATOM), double contrast was performed according to Reynolds and uranyl acetate. Electron microscopic research was carried out using a TEM-100 transmission electron microscope. Photo documentation using a SONY-H9 digital camera. Semi-thin sections with a thickness of 1-2 μm were made on an LKB-3 ultramicrotome (Sweden). They were stained with methylene blue.

Morphometric studies were performed after four weeks of the experiment on histological preparations stained with hematoxylin and eosin, using VideoTest-5.0, KAARA Image Base, Stepanizer and Microsoft Excel programs on a personal computer. Statistical processing of digital data was performed with the help of the "Excel" software using the parametric method.

Results

As the research results obtained by us showed, the structure of the mesenteric lymph nodes in animals of the intact group corresponded to the species norm. The morphological structure of nodes in the group of control male and female rats corresponded to the norm for the given age of the animal, and no deviations from the norm were observed at the end of the research. Lymph nodes are surrounded by a connective tissue capsule, which gives cortical and medullary trabeculae to the thickness of the parenchyma of the organ. Vessels and nerves pass through the latter. There is a gate on the concave part of the node. The parenchyma is built from the cortex of the node, located on the periphery, and the more centrally located medulla. The cortex contains primary and secondary lymphoid nodules. The medulla, in turn, consists of medullar sinuses and medullar cords (Fig. 1).

The lymphoid component of the parenchyma of mesenteric lymph nodes is formed by lymphocytes, macrophages, and plasma cells. Lymphocytes are divided into small, medium and large. Reticular cells and tissue form a three-dimensional framework that is filled with lymphoid cells. Electron microscopy established that all cells have a typical structure. Small lymphocytes with a diameter

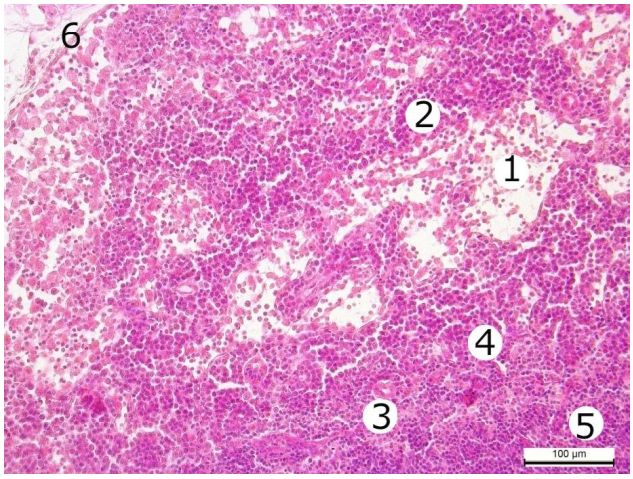

Fig. 1. A fragment of a mesenteric lymph node of an intact female white rat. Coloring with azan. Magnification: x200. Conventional signs: 1 - medullar intermediate lymphatic sinus; 2 - medullar cord; 3 - capillary venule; 4 - paracortical area; 5 - cortex; 6 - lymph node capsule.

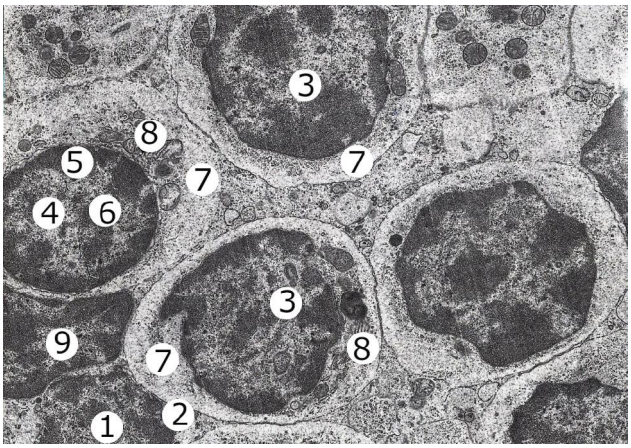

Fig. 2. Submicroscopic structure of the mesenteric lymph node cortex of a white female rat of the intact group. Electron micrograph. Magnification: x6000. Conditional signs: 1 - the nucleus of a small lymphocyte; 2 - cytoplasm of a small lymphocyte; 3 - the nucleus of an average lymphocyte, 4 - euchromatin in the nucleus of an average lymphocyte; 5 - heterochromatin; 6 - nucleolus; 7 - cytoplasm of an average lymphocyte; 8 - mitochondria; 9 - nucleus of a dendritic cell.

of 6-7 microns contain an oval large nucleus, which is surrounded by a thin rim of cytoplasm containing unchanged organelles (Fig. 2). Medium lymphocytes with a diameter of 7-9 μm have a rounded nucleus. Cytoplasm contains ribosomes, granular endoplasmic reticulum and mitochondria. Lymphoblasts with a diameter of about 10 μm are distinguished by the fact that their nucleus contains more euchromatin than heterochromatin, which makes them appear lighter.

After four weeks of daily exposure to monosodium glutamate, when studying the histological structure of mesenteric lymph nodes of male and female rats, it was found that the capsule of the organ thickens, which is associated with an increase in blood vessels in it, an increase in the proportion of adipose tissue, and the appearance of vacuole-like structures. An increase in the number of secondary lymphoid nodules in the nodule cortex was noted, which indicates the immune activity of this organ (Fig. 3). Germinal centers are clearly visualized, somewhat elongated in shape. The cortex and medulla layers are also moderately thickened.

The cortex sinuses and the marginal sinus are not expanded. The medullar sinuses are tortuous and narrowed. Medullar cords are thickened, contain densely located B-lymphocytes, plasma cells and macrophages (Figs. 3, 4). No differences in the histological structure of the mesenteric lymph nodes of male and female rats were found after four weeks of monosodium glutamate exposure. The paracortical area contains a greater number of post-capillary venules with a high endothelium compared to the intact and control groups of animals.

Changes in the vascular bed are observed, in particular, the arteries both in the thickness of the organ and in its gates contain a thickened wall, their lumen is full of blood. Veins are also full-blooded, dilated and deformed.

Using the morphometric method, it was established that the relative area of the mesenteric lymph node cortex after four weeks of the experiment decreases compared to the intact group of animals by only 1.95 % in male rats and by 4.21 % in female rats. The relative area of the node medulla increases by 3.06 % and 6.65%, respectively. The cortico-medullar index is 5.09 % ($p<0.05$) and 10.06 % ($p<0.001$) significantly less than the indicator of the intact group of animals (tables 1, 2).

The relative area of lymphoid nodules increases in comparison with the intact group of animals by 4.84 % in

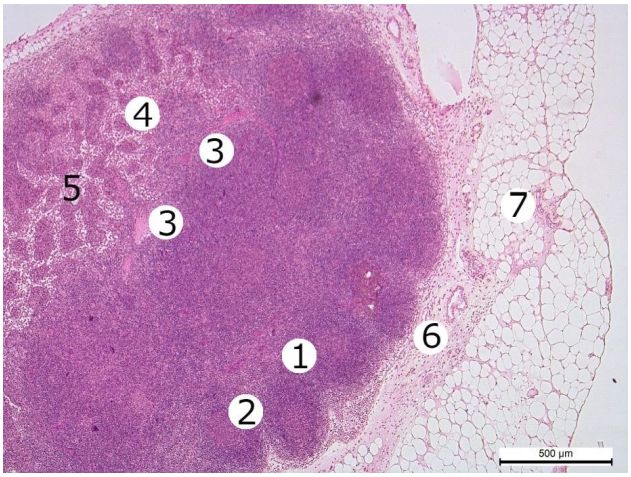

Fig. 3. A fragment of a mesenteric lymph node from a male white rat after four weeks of MSG exposure. Staining with hematoxylin and eosin. Magnification: x50. Conventional signs: 1 - secondary lymphoid nodule; 2 - cortical intermediate lymphatic sinus; 3 - post-capillary venules in the cotrex; 4 - thickened medullary cord; 5 - narrowed medullary sinus; 6 - thickened capsule; 7 - fatty tissue around the organ.

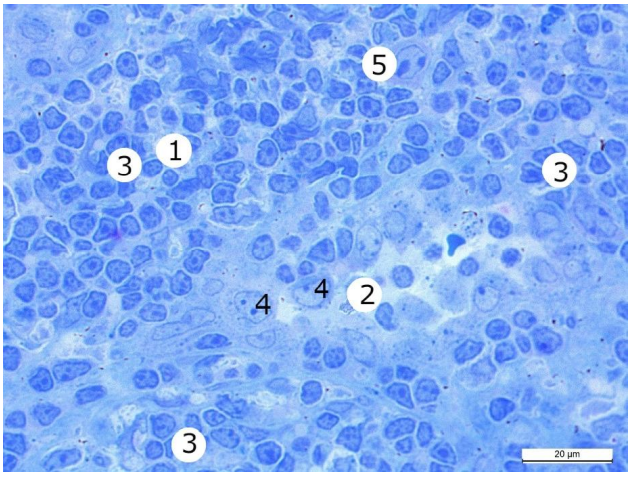

Fig. 4. A fragment of a mesenteric lymph node medulla of a female white rat after four weeks of monosodium glutamate exposure. Semi-thin cut. Staining with methylene blue. Magnification: x1000. Conventional signs: 1 - medullary cord; 2 - medullary sinus; 3 - small and medium lymphocytes; 4 - reticuloendotheliocytes; 5 - lymphoblast.

Table 1. Morphometric parameters of the structural components of the mesenteric lymph nodes of the studied male rats (M±m).

Parameter, units of measurement	Group of animals		p
	Intact	Experimental	
The relative area of the mesenteric lymph node cortex, %:	61.08±1.56	59.89±1.31	p>0.05
- lymphoid nodule	45.28±1.19	43.09±1.01	p<0.05
- germinal center	8.109±0.128	10.82±0.29	p<0.001
- mantle	37.17±0.97	32.27±0.94	p<0.001
- subcapsular sinus	4.389±0.219	4.519±0.161	p>0.05
- cortical sinus	4.031±0.159	4.131±0.122	p>0.05
- paracortical area	7.379±0.292	7.983±0.251	p<0.05
The relative area of the mesenteric lymph node medulla, %:	38.92±0.78	40.11±0.61	p>0.05
- medullary cord	18.71±1.01	21.33±0.76	p<0.05
- medullary sinus	20.41±1.15	18.78±0.69	p<0.05
Cortico-medullar index	1.571±0.112	1.491±0.11	p<0.05

Table 2. Morphometric parameters of the structural components of the mesenteric lymph nodes of the studied female rats (M±m).

Parameter, units of measurement	Group of animals		p
	Intact	Experimental	
The relative area of the mesenteric lymph node cortex, %:	61.23±1.70	58.65±1.43	p<0.05
- lymphoid nodule	45.37±1.22	42.2±1.21	p<0.05
- germinal center	8.152±0.109	10.69±0.24	p<0.001
- mantle	37.22±0.89	31.51±0.87	p<0.001
- subcapsular sinus	4.409±0.191	4.511±0.109	p>0.05
- cortical sinus	4.041±0.133	4.022±0.133	p>0.05
- paracortical area	7.412±0.269	7.923±0.332	p<0.05
The relative area of the mesenteric lymph node medulla, %:	38.77±0.76	41.35±0.63	p<0.05
- medullary cord	18.65±1.05	21.62±0.83	p<0.05
- medullary sinus	20.12±1.04	19.73±0.75	p>0.05
Cortico-medullar index	1.581±0.109	1.422±0.178	p<0.001

male rats and by 6.99 % (p<0.05) in female rats. The relative area of the germinal center increases by 33.42 % and 31.17 % (p<0.001). The mantle zone decreases by 13.18 % and 15.34 %, respectively (see Tables 1, 2).

The relative area of the marginal lymphatic sinus increases compared to the intact group of animals by 2.96 % in male rats and by 2.27 % in female rats. The relative area of cortical intermediate lymphatic sinuses increases in male rats by 2.48 % and decreases in female rats by 0.47 %. The relative area of the cortical area increases and is, respectively, 8.13 % and 6.88 % significantly more (p<0.05) than the indicator of the intact group of animals. The relative area of the medullar intermediate lymphatic sinuses decreases and is 7.99 % (p<0.05) and 1.94 % (p>0.05) less than the parameters of the intact group of animals. The relative area of medullar cords increases and is, respectively, 14.01 % and 15.92 % significantly more (p<0.05) than the parameters of the intact group of animals (see Tables 1, 2).

During the electron microscopic examination of the mesenteric lymph nodes of the experimental group of animals, it was found that the intercellular spaces are expanded, often containing vacuole-like structures. Part of lymphocytes of all populations with signs of apoptosis (Fig. 5). The other part has an uneven contour of the karyolemma, the nucleolus is not visualized in all

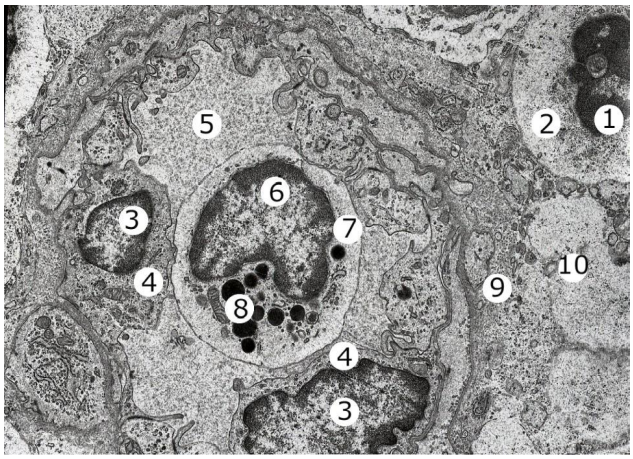

Fig. 5. Fragment of a mesenteric lymph node medulla of a white male rat after four weeks of exposure to monosodium glutamate. Electron micrograph. Magnification: x6000. Conventional signs: 1 - lymphocyte karyolysis; 2 - organelle-free cytoplasm of a lymphocyte; 3 - endotheliocyte nucleus, 4 - endotheliocyte cytoplasm, 5 - venule lumen; 6 - monocyte nucleus, 7 - monocyte cytoplasm, 8 - osmiophilic inclusions, 9 - swollen and thickened basement membrane, 10 - perivascular edema.

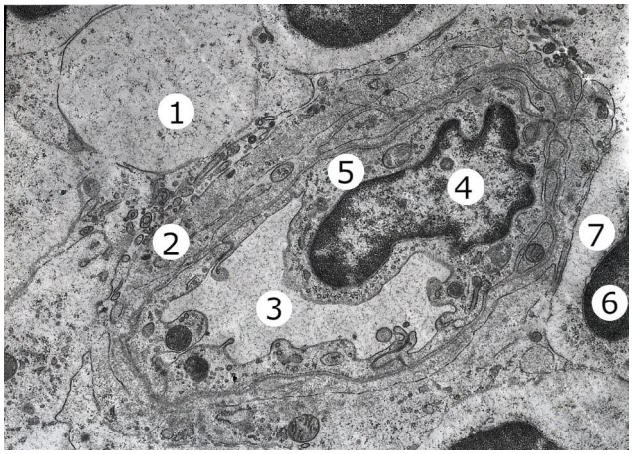

Fig. 6. Blood capillary in the cortex of a mesenteric lymph node of a white female rat after four weeks of MSG exposure. Electron micrograph. Magnification: x8000. Conventional signs: 1 - severe perivascular edema; 2 - stratified basement membrane; 3 - narrowed, deformed hemocapillary lumen; 4 - deformed, swollen endotheliocyte nucleus in the vessel wall; 5 - endotheliocyte cytoplasm; 6 - lymphocyte nucleus, 7 - illuminated cytoplasm.

lymphocytes, the cytoplasm is clear and contains organelles. Mitochondria are hypertrophied with a light matrix.

Perivascular edema is observed. The basement membrane of hemocapillaries, venules, and arterioles is thickened, stratified, and their lumen is somewhat narrowed. Endotheliocyte nuclei in the vessel wall are somewhat swollen, the lumenal surface of the plasmalemma forms numerous microvilli, which actually narrow the lumen of the vessel (Fig. 6). Organelles in the cytoplasm of endotheliocytes lose their contours.

Discussion

So, after four weeks of sodium glutamate action on histological preparations of mesenteric lymph nodes, intensive formation of germinal centers, increased proliferation and differentiation of lymphoid cells is observed. These are signs of antigenic stimulation. There is an immunoinducing effect with increased proliferation of activated lymphocytes and their subsequent differentiation into plasma cells. This is the morphological prerequisite for increased synthesis of immunoglobulins.

We believe that all changes in this term of the experiment are the primary reaction of immune organs to the daily administration of monosodium glutamate, similar changes have been described under the influence of other factors [15, 16]. Changes in the vascular bed are non-specific for this organ. Signs of edema of the parenchyma of lymph nodes are that the intercellular space is expanded and contains vacuole-like structures [16].

From the studies described in the literature, it is known that the administration of monosodium glutamate in a dose of 30 mg/kg of body weight leads to the accumulation of an excessive amount of substances of low and medium molecular weight in the body and a decrease in the ability of the kidneys to excrete toxic products. The shift of markers of intoxication syndrome towards catabolic substances was revealed. After a week of the experiment, the results correspond to the phase of partial compensation, characterized by an increased concentration of substances of low and medium molecular weight in erythrocytes and blood plasma. After two weeks and up to one month of the experiment, catabolic markers of endogenous intoxication prevail, which continue to accumulate in erythrocytes and plasma, which indicates the transition to the phase of partial decompensation of all systems and organs involved in detoxification [12]. Based on these results, the organs of the immune system are affected by intoxication of the body, as a side effect of monosodium glutamate.

Similar changes were found in other lymphoid organs under the influence of a high-calorie diet. An increase in the number of lymphocytes in the thymus parenchyma was found in rats. In the spleen of rats, the volume fraction of white pulp decreased, the number of cells in it also decreased, and the mass of the spleen increased. On the part of the vessels, hematuria and swelling of the parenchyma in both the thymus and the spleen were found, which once again confirms the non-specificity of changes in the vascular bed. The author explains the above changes as a manifestation of the body's general adaptive response [18].

As is well known, the population of "naïve" T-cells (helpers) decreases quantitatively with age, which leads to deterioration of the immune system. In animal model studies, high-calorie diet and obesity compromise the immune system through effects on T-cells due to increased adipogenesis in primary lymphoid organs and systemic inflammation in general. Given the fact that obesity increases the risk of many age-related diseases, impaired immune competence is a possible link between obesity and the development of diseases in the elderly and senile [5].

The results of a study in which experimental animals were fed a high-calorie diet consisting of 20 % protein, 20 % carbohydrates and 60 % fat for two weeks indicate that the proportion of macrophages and stromal cells that contain lipid inclusions increases in the parenchyma of lymph nodes. For comparison, a group of animals was used that received a low-calorie diet that included 20 % protein, 70% carbohydrates and 10 % fat for two weeks. The authors proved that stromal cells express a large number of genes related to lipid metabolism, which suggests that lymph nodes are involved in lipid metabolism [15]. The presence of lipid inclusions in the organs of the immune system is described in obesity.

Changes at the submicroscopic level were found in the lymph nodes during long-term use of the opioid nalbuphine. The authors describe that the six-week and eight-week administration of the opioid nalbuphine leads to pathological changes in the lymph nodes at the submicroscopic level: the number of lymphoblasts with signs of mitosis decreases, the number of lymphocytes with significant nuclear changes and damaged organelles in the cytoplasm of cells increases, which leads to the appearance of lymphoid cells with phenomena of preapoptosis and apoptosis, the intercellular spaces increase [16].

From the study conducted by the authors, it is known that the addition of monosodium glutamate to the diet of rats causes a delay in the removal of Na, K and water from the body. This, in turn, causes arterial hypertension [4].

Conclusions

It was found that even short-term daily exposure of male and female rats of reproductive age to monosodium glutamate, namely four weeks, causes changes in the structural organization of mesenteric lymph nodes. Swelling of the parenchyma of nodes and signs of immune activity are observed. There is a significant decrease in the relative area of lymphoid nodules and the mantle zone, an increase in the relative area of the medulla of the node, cortico-medullar index, germinal centers, paracortical area, and medullar cords. Arteries with a thickened wall are full-blooded, veins are deformed full-blooded. Submicroscopically, the proportion of apoptically changed cells increased.

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ЗМІНИ СТРУКТУРНОЇ ОРГАНІЗАЦІЇ ЛІМФАТИЧНИХ ВУЗЛІВ ПРИ КОРОТКОТРИВАЛІЙ ДІЇ ГЛУТАМАТУ НАТРІЮ

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Глутамат натрію є поширеною харчовою добавкою, котра відноситься до групи підсилювачів смаку, які використовують у широкому асортименті продуктів харчової промисловості. Потенційно негативний вплив глутамату натрію на здоров'я людини спонукає поставити під сумнів безпеку його широкого застосування. В статті наведено та проаналізовано дані експериментального дослідження, проведеного на 40 білих щурах-самцях і самках репродуктивного віку (2,5-3,5-місячних) масою 120-190 г. Мета дослідження - вивчити гістологічні, морфометричні та ультраструктурні зміни лімфатичних вузлів щурів в умовах короткотривалого впливу глутамату натрію. Експериментальній групі тварин (10 щурів-самців, 10 щурів-самок), котра перебувала на стандартному харчовому раціоні віварію, впродовж чотирьох тижнів щодня в один і той же час піпеткою додавали глутамат натрію в дозі 0,07 г/кг маси тіла щура з подальшим вільним доступом до води та їжі. У тварин інтактної групи будова брижових лімфатичних вузлів відповідала видовій нормі. В експериментальній групі тварин через чотири тижні дії глутамату натрію виявлено, що прикіркова ділянка містила більшу кількість закапілярних венул з високим ендотелієм порівняно з інтактною та контрольними групами тварин. Встановлено зміни в судинному руслі, зокрема, артерії як в товщі органа, так і в його воротах містять потовщену стінку, їх просвіт повнокровний. Вени

також повнокровні, розширені та деформані. Спостерігається набряк паренхіми вузлів та ознаки імунної активності. Відбувається достовірне зменшення відносної площі лімфоїдних вузликів та плащової зони, збільшення відносної площі мозкової речовини вузла, кірково-мозкового індексу, зародкових центрів, прикіркової ділянки, мозкових тяжів. Частина лімфоцитів всіх популяцій з ознаками апоптозу. Інша частина має нерівний контур каріолеми, ядерце візуалізується не у всіх лімфоцитах, цитоплазма просвітлена, містить органели. Мітохондрії гіпертрофовані зі світлим матриксом. Отже, навіть короткотривалий щоденний вплив на організм глутамату натрію, а саме чотири тижні, викликає зміни структурної організації брижових лімфатичних вузлів.

Ключові слова: *експеримент, глутамат натрію, ліматичний вузол, кіркова речовина, мозкова речовина, лімфоцити.*