Electron microscopic changes of lymph nodes during correction of sodium glutamate action by melatonin

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Introduction

Today, more than 2,500 additives are deliberately added to foods to preserve their properties and extend their shelf life. One of the most widely used additives both in Ukraine and around the world is monosodium glutamate [12, 19]. It increases appetite and enhances the taste of foods, which leads to an increase in the amount of food consumed per day, causing a high-calorie diet as in this experiment. Excess energy in the body leads to metabolic disorders, overweight and, as a result, obesity [3]. Despite its widespread use in the food industry, some questions about its effects on the body remain unanswered [20].

The literature describes studies performed on animals using this additive. Sodium glutamate is known to be neurotoxic, able to provoke degeneration of neuronal populations, and its effects on the body are accompanied by the development of pathological conditions such as stroke, epilepsy, schizophrenia, anxiety, depression, Parkinson’s disease, Alzheimer’s disease, Huntington’s disease and Lou Gehrig’s disease [19].

Metabolic characteristics of sodium glutamate are the development of insulin resistance, diabetes mellitus, low levels of high-density lipoprotein in the blood, high levels...
of triglycerides, signs of inflammation and general oxidative stress [6, 8, 13]. It is known that hyperinsulinemia and metabolic disorders are directly related to reduced life expectancy, which is regarded not only as a medical but also a social problem.

However, the effect of monosodium glutamate on lymphoid organs remains insufficiently studied. It is known that the organs of the immune system are the rod that provides homeostasis, the body's resistance to foreign agents. Lymph nodes belong to the secondary immune organs, where antigen-dependent proliferation and differentiation of T and B lymphocytes occurs.

Also no less relevant is the issue of correction of changes caused by the action of monosodium glutamate. The drug chosen for correction is a synthetic analogue of melatonin (N-acetyl-5-methoxytryptamine) - a hormone of the pineal gland, an important regulator of sleep and circadian rhythms, which is synthesized by pinealocytes of the pineal gland under the control of the suprachiasmatic nucleus of the hypothalamus [7, 10, 11, 15]. Recently, the number of studies on the possible effects of this substance is growing [1, 14, 17, 18]. The neuroimmunomodulatory effect of melatonin on the immune system is supported by the presence of specific melatonin receptors in the immune system, as well as immunocompetent cells. These receptors are located both in the plasma membrane and in the cell nucleus. The antioxidant properties of melatonin and its effect on neutrophil infiltration have been studied in numerous experimental models in animals [4].

The purpose of the study: to study the electron microscopic changes in the parenchyma of the lymph nodes of rats in the correction of the action of monosodium glutamate by melatonin.

**Materials and methods**

This experimental study was performed on 66 white male and female rats of reproductive age (2.5-6.5 months) weighing 120-250 g.

The structure of mesenteric lymph nodes of white rats under the physiological norm at the electron microscopic level was studied in 10 intact animals. The experimental animals were divided into 4 groups: the first group (10 animals), which were fed a high-calorie diet (HCD) for six weeks by adding monosodium glutamate; the second group (10 animals), which were fed HCD for six weeks, then transferred to the standard diet of vivarium and melatonin was administered for two weeks; the third group (10 animals) and the fourth group (10 animals) were the same as the previous one, but melatonin was used for four and six weeks, respectively. There were 5 male rats and 5 female rats in each group. HCD was achieved by adding to the diet of monosodium glutamate at a dose of 0.07 g/kg body weight of rats. The dose of melatonin was 10 mg/kg body weight of rats, administered orally daily at the same time in the afternoon.

The control was 16 white rats, which instead of HCD received a standard diet of vivarium.

All experimental animals were kept in the vivarium of Lviv National Medical University named after Danylo Halytsky. The research was conducted in accordance with the provisions of the European Convention for 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 № 3447 - IV "On the Protection of Animals from Cruel behavior ", general ethical principles of animal experiments, approved by the First National Congress of Ukraine on Bioethics (2001).

Before collecting the material, the animals were anesthetized with anesthesia with diethyl ether. Fixation of pieces of lymph nodes was performed with a 1.5 % solution of osmium tetroxide in 0.2 M sodium cacodylate solution at pH 7.2 for 2-2.5 hours in the cold. Dehydration in increasing concentrations of ethyl alcohol (50°, 70°, 90° and absolute) for 30 minutes each and propylene oxide for 10 minutes. The material was poured into a mixture of epoxy resins and polymerized for 24 h in a thermostat at 60°C. Sections were made on an ultramicrotome UMT-6M with a diamond knife (DIATOM) and double contrast was performed according to Reynolds and uranyl acetate. Submicroscopic examinations of the organ were performed using an electron transmission microscope TEM-100. The test material was documented using a SONY-H9 digital camera.

**Results**

The electron microscopic structure of the mesenteric lymph nodes of male and female rats of reproductive age of the intact and control groups corresponds to the species norm. Externally, the organ is surrounded by a connective tissue capsule, from which the cortical and medulla parts begin, which penetrate the parenchyma of the node. On the periphery is the cortical substance, which consists of primary and secondary lymphoid nodules, cortical intermediate lymphatic sinuses. Under the capsule there is the marginal sinus, which extends into the cortical intermediate lymphatic sinuses. Central and closer to the gate of the node there is the medulla, which is built of medulla cords and medulla intermediate lymphatic sinuses. In the area of transition of the cortical substance to the medulla there is the paracortical zone, which belongs to the T-dependent zone. The lymphoid tissue of the node is represented by small, medium and large lymphocytes. The skeleton of the organ is formed by reticular cells and tissue. Among lymphocytes are plasma cells and macrophages. The walls of the intermediate sinuses are lined with reticuloendothelialocytes, or shore cells. Small lymphocytes have a typical structure, their size is 6-7 μm, a relatively large nucleus is surrounded by a thin strip of cytoplasm. Medium lymphocytes have a rounded nucleus, which contains both heterochromatin and euchromatin, their size is 7-9 μm, organelles are located in the cytoplasm. Large lymphocytes (lymphoblasts) also have a typical structure, the nucleus contains mainly euchromatin, so it is lighter...
than other lymphocytes, their size is about 10 μm, karyolemma is smooth, light cytoplasm filled with organelles. Plasmocytes have a nucleus with a specifically located heterochromatin, which resembles a "wheel spoke". The eccentrically located nucleus separates the cytoplasm. The parenchyma of the lymph node contains vessels of the hemomicrocirculatory tract. The arteries that enter the cortical trabeculae divide into hemocapillaries, which merge into the capillary venules, most of which are located in the paracortical area (Fig. 1). The latter, in turn, merge into the veins and exit through the gate of the node.

Submicroscopically, after six weeks of HCD, the number of apoptically altered lymphocytes increases. The part of lymphocytes that does not show signs of karyorrhexis or karyolysis has a karyolemma with deep intussusception, the cytoplasm is enlightened, organelles have signs of damage. The number of macrophages and plasma cells increases in the parenchyma of the node. Their cytoplasm contains numerous primary and secondary lysosomes, including fragments of destroyed lymphocytes and osmophilic (fat) inclusions (Fig. 2), which are signs of alimentary obesity. The tubules of the granular endoplasmic reticulum in cells with signs of edema are dilated. Mitochondrial ridges are swollen, damaged, with an enlightened matrix. The nuclei of reticuloendotheliocytes are enlarged and deformed, their processes are thickened and swollen. Vessels of a hemomicrocirculatory channel also undergo changes at the level of all links. The wall of arteries and arterioles is thickened, the lumen is filled with shaped elements of blood. Hemocapillaries have a thickened basement membrane, endothelial cell nuclei are deformed and enlarged, and the luminal surface of its cytoplasma forms numerous intussusceptions and depressions (Fig. 2). Through defects in a wall of blood capillaries are observed. Venules and veins with dilated full-blooded lumen.

Electron microscopically in the second group of experimental animals, all detected changes are similar to the previous group. A significant proportion of the cellular composition of the parenchyma of the lymph node is occupied by apoptically altered lymphocytes, macrophages and plasma cells (Fig. 3). The intercellular space is expanded, there are signs of perivascular edema, a large number of osmophilic (fatty) inclusions are in the intercellular space and in the cytoplasm of macrophages and plasma cells. All lymphatic sinuses are dilated, a large number of collagen fibers and microfibrils in the parenchyma of the node compared with the intact group of animals.

In the third group of experimental animals, electron microscopically in the parenchyma of the lymph nodes revealed that the proportion of destructive changes decreased slightly compared to previous experimental groups. The karyolemma of lymphocyte nuclei has uneven contours, with numerous depressions and protrusions, their cytoplasm is somewhat enlightened (Fig. 4). The number of macrophages and plasma cells remains high. Reticuloendotheliocytes have thickened processes. Arteries and arterioles with a thickened wall, full-blooded. Veins and venules with dilated, deformed lumen. The lumen of hemocapillaries is narrowed, the basement membrane is thickened, swollen.

In the fourth group of experimental animals, after six weeks of correction of the action of HCD by melatonin, electron microscopically in the parenchyma of the lymph nodes revealed that among the unaltered lymphocytes there are destructively altered cells. Marginal, cortical and...
medulla intermediate lymphatic sinuses are somewhat dilated. The number of macrophages and plasma cells, compared with the previous group of animals, decreased. In some lymphocytes the nucleolus is not clearly expressed, the karyolemma is not equal, the cytoplasm is enlightened (Fig. 5). The number of osmophilic (fat) inclusions decreased both in the intercellular space and in the cytoplasm of cells, which indicates the regression of signs of alimentary obesity.

Discussion

The literature describes a study with the introduction of rat glutamate sodium at a dose of 30 mg/kg body weight for 30 days, noted the production in animals of excessive amounts of low and medium molecular weight and decreased excretory capacity of the kidneys. Low and medium molecular weight substances include creatinine, urea, oligosaccharides, lactic acid, bilirubin, amino acids, cholesterol, lipid peroxidation products and other compounds [12].

The study was performed on pregnant rats, which were divided into three groups - control, a group of animals that received monosodium glutamate with food, and a group that received a high-calorie diet due to the caloric content of food consumed. It was concluded that pregnant rats who consumed monosodium glutamate had a significant increase in body mass index, which the authors associated with the development of leptin resistance. Moreover, obesity in pregnant rats caused by monosodium glutamate had a greater effect on offspring metabolism and body weight than obesity induced by a high-calorie diet [2].

In an experimental study, administration of sodium glutamate to female rats at doses of 2 and 4 mg/kg body weight caused obesity in newborns. The authors studied metabolic changes at the ages of 4, 8, 12, 16 and 20 months. At a young age (four months), the level of Li index, triglycerides, total cholesterol, TNF-α and transaminases increased. While adiponectin levels decreased, glucose tolerance and insulin sensitivity were markedly altered. However, from 16 months of age, the level of Li and TNF-α index decreased significantly, and adiponectin increased, glucose and insulin homeostasis was restored. Obesity has been shown to be a major contributing factor to
premature metabolic changes in rats, however, in older age, all changes are offset [9].

A study in newborn rats treated subcutaneously from day 2 to day 12 of life with monosodium glutamate at a dose of 4 mg/kg/day was described. The correction was performed from 30 days of life with quercetin at a dose of 75 mg/kg/day. It is concluded that quercetin successfully improves metabolic changes caused by exposure to sodium glutamate. In addition, quercetin normalized glucose levels and minimized toxic effects associated with monosodium glutamate on liver and kidney function. These effects are associated with the antioxidant properties of quercetin [16].

The results of a study conducted on eight-week-old rats on a high-calorie diet and melatonin correction for ten weeks at a dose of 1 mg/kg/day showed that melatonin supplementation reduced serum triglycerides, total cholesterol, low lipoprotein protein and weight gain by reducing the level of lipogenesis and increasing the lipolytic capacity of adipocytes. Thus, the authors concluded that melatonin can be considered a potential therapeutic agent for reducing metabolic and inflammatory disorders caused by obesity [5].

The novelty of the results described by us is the use of electron microscopic research methods, which gave new data at the ultrastructural level on the structure of lymph nodes under the action of monosodium glutamate and its correction by melatonin.

Conclusions

1. Electron microscopic examination showed that monosodium glutamate causes changes in the parenchyma of the lymph nodes as in alimentary obesity. After six weeks of HCD in the parenchyma of the lymph nodes there are profound destructive changes in the cellular composition of the organ, violations at the level of all parts of the vascular bed.

2. The introduction of melatonin leads to a significant restoration of the structural organization and, consequently, the function of this organ.

References


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