

ORIGINAL ARTICLE

HISTOLOGICAL AND MORPHOLOGICAL CHANGES IN THE LYMPHOID STRUCTURES OF THE GASTRIC MUCOUS MEMBRANE IN WHITE RATS WITH THE ADMINISTRATION OF SODIUM GLUTAMATE

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ABSTRACT

The aim: To determine the histological and morphological changes of the lymphoid structures of the stomach in male rats under the influence of oral sodium glutamate at the rate of 15 mg/kg of body weight.

Materials and methods: The scientific experiment was performed on 20 white non-linear male rats of reproductive age (4-5 months). The experimental animals were divided into two groups (10 rats in each group), which were orally received monosodium glutamate at a dose of 15 mg/kg body weight every day. We studied the effect of 2 and 4 weekly administration of monosodium glutamate at a dose of 15 mg/kg body weight, respectively, in the I and II groups of experimental animals (depending on the week of their decapitation). Rats of the control groups (n=10) were injected with a placebo for 2 and 4 weeks, namely 0.5 ml of dechlorinated tap water at room temperature. Intact control animals were also divided into two groups, 5 rats each, depending on the week of decapitation: respectively, III group – decapitation on the 2nd week of the experiment; IV group - decapitation on the 4th week of the experiment. After the experiments were completed, animals were decapitated under light ether anesthesia. According to the purpose of the study, pieces of rat stomach measuring 1.0 x 1.0 cm were taken from the front wall of the bottom of the stomach near the great curvature, cardiac and portal parts of the organ. Histological preparations were examined using a MICROmed SEO SCAN light microscope and a Vision CCD Camera. Morphometric studies were carried out according to the method of S. B. Stefanov, using grids No. 3/16. For electron microscopic examination, pieces of the stomach wall of rats were fixed in a 2.5% solution of glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2-7.4) with subsequent fixation in a 2.0% solution of osmium tetroxide. After dehydration in alcohols and acetone, the material was embedded in eponaraldite. Sections were made on an LKB-8800-III ultramicrotome and studied using a JEM - 100-V microscope. To study the structural components of the lymphoid formations of the mucous membrane of different parts of the stomach of rats, semi-thin sections were made for the purpose of sharpening the blocks, which were stained with methylene blue.

Results: The analysis of the obtained data of the conducted experiment indicates that the administration of monosodium glutamate in a dose of 15 mg/kg of body weight to rats already after 14 days leads to an increase in the density and size of the lymphoid structures of the GMM. The number of immunocompetent cells between the fundus of the gastric glands and the muscle plate increases in the diffuse lymphoid tissue of the gastrointestinal tract of rats in all its parts, both in the I and II groups of experimental animals. These changes are most pronounced in the cardiac and portal parts of the stomach. In both groups of experimental animals, the migration of interepithelial lymphocytes, macrophages, plasma cells, and tissue basophils to the surface epithelium increases. In both groups of experimental animals (and the II group of rats), lymphoid nodules and lymphoid pre-nodules of the gastric mucous membrane (GMM) are located between the bottom of the gastric glands and the muscular plate of the GMM. A gradual increase of medium lymphocytes in the GMM was established both in animals of I and II groups, while large lymphocytes increased in almost the same amount in experimental animals of both groups. Similar changes occur in the characteristics of the number of plasma cells, macrophages and tissue basophils in the lymphoid pre-nodules of GMM.

Conclusions: Administering monosodium glutamate to rats at a dose of 15 mg/kg of body weight for 2 weeks leads to an increase in the density and size of lymphoid structures of the mucous membrane in all parts of the stomach with a predominant increase in the number of immunocompetent cells between the bottom of the gastric glands and the muscle plate. At the same time, more pronounced changes were found in the number of small lymphocytes, which tend to decrease by the 2nd week of the experiment, and vice versa – their density increases by the 4th week of monosodium glutamate administration.

KEY WORDS: monosodium glutamate, stomach, lymphoid structures

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INTRODUCTION

Mucosa Associated Lymphoid Tissue or MALT are dispersed aggregates of non-encapsulated organized lymphoid tissue in the mucosa that are associated with

local immune reactions on the surface of the mucosa. There are three main types of MALT - this is lymphoid tissue associated with the intestine (GALT – Gut Associated Lymphoid Tissue), lymphoid tissue associated

with the bronchi (BALT – bronchus associated lymphoid tissue) and lymphoid tissue associated with nasal part of the pharynx (NALT – nasal associated lymphoid tissue). MALT determines the effectiveness of the immune defense system of the mucous membranes of internal organs [1]. The study of lymphoid formations of the mucous membranes, including the stomach, is an extremely urgent task of both clinical and experimental research, which is due to the fact that in recent years the pollution of the external environment by toxic and harmful substances, in particular, antigens, and as well as products and components of food products that enter a person's stomach with food.

Various experimental and clinical studies are conducted to determine the impact of food additives on health. The most common of them is monosodium glutamate. The food supplement, which has been known as a "taste enhancer" for more than 100 years, is the fifth type of taste in Japan – "umami", after sour, bitter, salty and sweet. This additive was first discovered by the Japanese scientist Professor Kikunae Ikeda and introduced into mass production as one of the most common food additives after salt and pepper [2]. Studies have shown that even minimal doses of monosodium glutamate (0.6 and 1.6 mg/g of body weight for two weeks or 100-500 mg/kg of body weight for three weeks) can cause harmful effects on the body of humans and laboratory animals, in particular rodents [3]. It was established that monosodium glutamate induced pronounced changes in the organs of the digestive system. Experimental feeding of monosodium glutamate to rats for 1-9 months at a dose of 2 mg/g of body weight caused characteristic histological changes in the pancreas, which were characterized by a decrease in the number of β -cells, hemorrhages, and signs of fibrosis [4].

Therefore, information about the negative impact of monosodium glutamate on the structural organization of organs, in particular the nervous, digestive, immune and other systems, is increasingly appearing in the professional literature [2]. The study of the peculiarities of histological and morphological changes in the lymphoid structures of the stomach against the background of sodium glutamate administration in the experiment can supplement knowledge about the effect of this food additive and the structural organization of the body's defense system on its administration.

THE AIM

The aim is to determine the histological and morphological changes of the lymphoid structures of the stomach in male rats under the influence of oral sodium glutamate at the rate of 15 mg/kg of body weight.

MATERIALS AND METHODS

The scientific experiment was performed on 20 white non-linear male rats of reproductive age (4-5 months) weighing 220-280 g, which were divided into two groups of 10 rats each. Experimental animals were kept in vivarium conditions in compliance with all regulations, namely the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), Council of Europe Directive 86/609/EEC (1986), the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Treatment", general ethical principles of experiments on animals, adopted by the First National Congress of Ukraine on Bioethics (2001).

The experimental animals were divided into two groups (10 rats in each group), which were orally received monosodium glutamate at a dose of 15 mg/kg body weight every day. Monosodium glutamate was dissolved in 0.5 ml of dechlorinated tap water at room temperature. We studied the effect of 2 and 4 weekly administration of monosodium glutamate at a dose of 15 mg/kg body weight, respectively, in the I and II groups of experimental animals (depending on the week of their decapitation).

Rats of the control groups (n=10) were injected with a placebo for 2 and 4 weeks, namely 0.5 ml of dechlorinated tap water at room temperature. Intact control animals were also divided into two groups, 5 rats each, depending on the week of decapitation: respectively, III group – decapitation on the 2nd week of the experiment; IV group - decapitation on the 4th week of the experiment.

All experimental animals were kept in the conditions of the vivarium of the Lviv National Medical University named after Danylo Halytskyi. Control and experimental animals were kept in separate boxes in the vivarium.

After the experiments were completed (after administration of monosodium glutamate on weeks 2 and 4 of the experiment), animals were decapitated under light ether anesthesia. Animals were weighed before decapitation. The skin and soft tissues of the abdomen were dissected, the abdominal cavity was cut, and the stomach of control and experimental animals was sampled. According to the purpose of the study, pieces of rat stomach measuring 1.0 x 1.0 cm were taken from the front wall of the bottom of the stomach near the great curvature, cardiac and portal parts of the organ. The obtained pieces of stomach preparations were fixed for 2 weeks in a 10.0% solution of neutral formalin, after which they were embedded in paraffin blocks. Histological preparations were examined using a MICROmed SEO SCAN light microscope and a Vision CCD Camera. Morphometric studies were carried out

according to the method of S. B. Stefanov, using grids No. 3/16. At the same time, the density (number) of cellular elements was determined in a large grid square on an area of 625 μm^2 in diffuse lymphoid tissue, lymphoid pre-nodules, and lymphoid nodules of the gastric mucous membrane (GMM). In lymphoid nodules, lymphoid pre-nodules, and diffuse lymphoid tissue, the number of small, medium, and large lymphocytes, plasma cells, macrophages, and tissue basophils was counted in 20 large squares of the 3/16 morphometric grid (the area of one large square is 625 μm^2). Subsequently, the average value of cell density in one large square was calculated.

For electron microscopic examination, pieces of the stomach wall of rats were fixed in a 2.5% solution of glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2-7.4) with subsequent fixation in a 2.0% solution of osmium tetroxide. After dehydration in alcohols and acetone, the material was embedded in eponaraldite. Sections were made on an LKB-8800-III ultramicrotome and studied using a JEM - 100-V microscope. To study the structural components of the lymphoid formations of the mucous membrane of different parts of the stomach of rats, semi-thin sections were made for the purpose of sharpening the blocks, which were stained with methylene blue.

The analysis and processing of the results of the examination of patients was carried out by the computer program Statistics 10.0 (StatSoftInc, USA) for Windows, using parametric and non-parametric methods of evaluating the obtained results.

RESULTS

It should be noted that in the control group of rats (III-IV groups) at different stages of experiment, we did not find any differences in the histological and morphometric evaluation of the lymphoid structures of the GMM. Therefore, in the future, the results of control animals of III and IV groups were combined into a single control group III (n= 10), with which the data of experimental animals of groups I and II at different stages of monosodium glutamate administration were compared.

The analysis of the obtained data of the conducted experiment indicates that the administration of monosodium glutamate in a dose of 15 mg/kg of body weight to rats already after 14 days leads to an increase in the density and size of the lymphoid structures of the GMM. The number of immunocompetent cells between the fundus of the gastric glands and the muscle plate increases in the diffuse lymphoid tissue of the gastrointestinal tract of rats in all its parts, both in the I and II groups of experimental animals. These changes are most pronounced in the cardiac and portal

parts of the stomach. In both groups of experimental animals, the migration of interepithelial lymphocytes, macrophages, plasma cells, and tissue basophils to the surface epithelium increases. In both groups of experimental animals (and the II group of rats), lymphoid nodules and lymphoid pre-nodules of the GMM are located between the bottom of the gastric glands and the muscular plate of the GMM.

The cellular composition of lymphoid formations of GMM in rats changes after administration of monosodium glutamate at a dose of 15 mg/kg of body weight. As shown in Table 1, two weeks after the introduction of monosodium glutamate in the lymphoid nodes of the GMM in rats, the number of small lymphocytes in all sections of the stomach significantly decreases compared to similar data in animals of the control group, and after 1 month after the introduction of a food supplement, on the contrary, it is significantly increases both in comparison with the data of the I group and with the indicators of the control rats of the III group.

Average lymphocytes significantly increased in both groups of experimental animals in lymphoid nodules of the SOS in all its parts. A similar trend was followed in the analysis of plasma cells. Large lymphocytes, on the contrary, in the lymphoid nodules of the GMM, on the 2nd week of taking monosodium glutamate, increased significantly in all parts of the stomach, with their gradual decrease until the 4th week of the experiment (rats of the II group) (Table I).

In an electron microscopic study, we established that when monosodium glutamate was administered to rats at a dose of 15 mg/kg of body weight, cytoplasmic outgrowths appeared in lymphocytes, which indicates their active migration. The penetration of lymphocytes through the basement membrane into the epithelial layer of the GMM is also determined, which indicates an increase in the number of interepithelial lymphocytes when sodium glutamate is administered.

The activity of immune reactions upon administration of monosodium glutamate is indicated by an increase in the number of macrophages in all lymphoid formations of in gastric mucous membrane (GMM) of experimental animals of groups I and II. A significant increase in macrophages was established in both groups I and II rats. At the same time, no significant difference was established between the data of the animals of the experimental groups, that is, the number of macrophages and tissue basophils actually remained the same both on the 2nd week and on the 4th week of the experiment. The ultrastructure of macrophages changes significantly from the 2nd week of the introduction of the food supplement, namely, the number of microvilli and pseudopodia on the plasmolemma increases, and the cytoplasm becomes heterogeneous,

Table I. Cellular composition of lymphoid nodules of the mucous membrane of the stomach of rats in the control and experimented groups

Cell type	Parts of the stomach	Groups of animals		
		I group	II group	III group
Small lymphocytes	Cardiac part	9.4±0.21**	12.04±0.16*+	11.16±0.23
	Bottom	10.44±0.28**	14.23±0.21*+	13.08±0.26
	Portal part	11.06±0.25**	16.45±0.18**+	14.02±0.21
Medium lymphocytes	Cardiac part	0.86±0.06	1.14±0.06**+	0.72±0.06
	Bottom	0.95±0.05	1.25±0.08*+	0.80±0.09
	Portal part	1.16±0.09	1.44±0.03*+	0.91±0.08
Large lymphocytes	Cardiac part	0.77±0.05*	0.63±0.07	0.51±0.07
	Bottom	0.75±0.07**	0.61±0.04*	0.42±0.05
	Portal part	0.50±0.06**	0.44±0.08*	0.28±0.07
Plasma cells	Cardiac part	1.61±0.09**	1.85±0.07**	0.80±0.06
	Bottom	1.82±0.10**	1.80±0.07**	0.92±0.10
	Portal part	2.28±0.12*	2.30±0.24*	1.21±0.07
Macrophagocytes	Cardiac part	1.69±0.07**	1.60±0.03**	0.68±0.05
	Bottom	1.40±0.06**	1.44±0.05**	0.60±0.07
	Portal part	2.20±0.08**	2.26±0.21**	0.84±0.11
Tissue basophils	Cardiac part	1.70±0.05**	1.77±0.05**	0.85±0.03
	Bottom	2.50±0.24**	2.54±0.06**	1.21±0.14
	Portal part	1.60±0.07**	1.72±0.05**	0.68±0.07

Note: the difference between the parameters of the rats of the experimental groups (I and II groups) and the control group (III group) is significant: * – p<0.05; ** – p<0.01; the difference between the indicators in rats of the I and II groups is significant: + - p<0.05.

Table II. Cellular composition of pre-lymphoid nodules of the gastric mucosa of rats in the control and experimental groups

Cells type	Parts of the stomach	Groups of animals		
		I group	II group	III group
Small lymphocytes	Cardiac part	8.12±0.18*	10.06±0.15*+	9.11±0.33
	Bottom	10.37±0.21*	12.80±0.17*+	11.42±0.26
	Portal part	10.70±0.23*	13.55±0.28*+	12.30±0.25
Medium lymphocytes	Cardiac part	0.85±0.07	1.08±0.06*	0.71±0.08
	Bottom	0.97±0.09	1.22±0.08*+	0.88±0.06
	Cardiac part	0.98±0.07	1.25±0.06*+	0.89±0.05
Large lymphocytes	Cardiac part	0.44±0.06	0.43±0.09	0.34±0.10
	Bottom	0.53±0.12	0.50±0.09	0.32±0.04
	Portal part	0.56±0.06	0.54±0.07	0.43 ±0.07
Plasma cells	Cardiac part	1.08±0.13*	1.11±0.06**	0.50±0.12
	Bottom	1.43±0.06**	1.41±0.10**	0.75±0.08
	Portal part	1.68±0.05*	1.73±0.06**	0.94±0.06
Macrophagocytes	Cardiac part	1.58±0.10**	1.54±0.05**	0.64±0.05
	Bottom	1.30±0.05**	1.22±0.07**	0.55±0.09
	Portal part	2.15±0.11**	2.14±0.09**	0.84±0.07
Tissue basophils	Cardiac part	1.60±0.04**	1.76±0.09**	0.86±0.07
	Bottom	2.21±0.14**	2.35±0.13**	1.14±0.12
	Portal part	1.77±0.06**	1.70±0.09**	0.85±0.08

Note: the difference between the parameters of the rats of the experimental groups (I and II groups) and the control group (III group) is significant: * – p<0.05; ** – p<0.01; the difference between indicators in rats of the I and II groups is significant: + - p<0.05.

Table III. Cellular composition of the diffuse lymphoid tissue of the gastric mucous membrane of rats in the control and experimental groups

Cell type	Parts of the stomach	Groups of animals		
		I group	II group	III group
Small lymphocytes	Cardiac part	3.94±0.26*	4.77±0.16*+	4.30±0.12
	Bottom	3.36±0.18*	4.08±0.24*+	3.70±0.16
	Portal part	4.30±0.21*	5.23±0.16*+	4.86±0.23
Medium lymphocytes	Cardiac part	0.65±0.08	0.84±0.04*+	0.54±0.09
	Bottom	0.77±0.09	1.06±0.05*+	0.65±0.08
	Portal part	1.34±0.06	1.50±0.08*	1.18±0.06
Large lymphocytes	Cardiac part	0.26±0.06*	0.25±0.04*	0.14±0.08
	Bottom	0.37±0.04	0.37±0.09	0.23±0.12
	Portal part	0.42±0.05	0.47±0.07*	0.27±0.06
Plasma cells	Cardiac part	1.11±0.09**	1.15±0.10**	0.52±0.08
	Bottom	1.15±0.06**	1.35±0.12**	0.66±0.07
	Portal part	1.30±0.05**	1.57±0.04**	0.72±0.05
Macrophagocytes	Cardiac part	0.44±0.10**	0.49±0.08**	0.21±0.04
	Bottom	0.60±0.08**	0.77±0.03**	0.22±0.07
	Portal part	1.05±0.08**	0.97±0.09**	0.37±0.06
Tissue basophils	Cardiac part	1.18±0.08**	1.26±0.09**	0.55±0.04
	Bottom	2.15±0.10**	2.21±0.08**	0.88±0.14
	Portal part	1.65±0.05**	1.69±0.07**	0.62±0.12

Note: the difference between the parameters of the rats of the experimental groups (I and II groups) and the control group (III group) is significant: * – $p < 0.05$; ** – $p < 0.01$; the difference between indicators in rats of the I and II groups is significant: + – $p < 0.05$.

containing many lysosomes, phagosomes, and pinocytotic vesicles. Macrophagocytes have various shapes and the cytoplasm contains many specific granules.

Tissue basophils are also significantly increased in animals of both experimental groups in all departments of lymphoid nodules of GMM. They migrate in the direction of the surface epithelium of the GMM.

In the 2nd week of the experiment, the number of small lymphocytes decreases in the lymphoid pre-nodules in comparison with such data in rats of the control group, while on the 4th week of administration of monosodium glutamate, on the contrary, a significant increase in them was found in all departments of the GMM (Table II).

A gradual increase of medium lymphocytes in the GMM was established both in animals of I and II groups, while large lymphocytes increased in almost the same amount in experimental animals of both groups. Similar changes occur in the characteristics of the number of plasma cells, macrophages and tissue basophils in the lymphoid pre-nodules of GMM.

The dynamics of changes in the density of cellular elements in the diffuse lymphoid tissue of GMM in rats is similar to the changes in lymphoid nodules and lymphoid pre-nodules (Table III).

On the 2nd week of sodium glutamate administration, the number of small lymphocytes in all parts of the

stomach in rats also decreases in the diffuse lymphoid tissue of the GMM. But after a 4-week introduction of the food supplement, its density begins to gradually increase, just as in the lymph nodes and pre-nodules of GMM. Average lymphocytes in the diffuse lymphoid tissue of GMM gradually increase with minimal changes in animals of the I group and maximum deviations from the data of the control group in the experimental animals of the II group. Large lymphocytes, plasma cells, macrophages, and tissue basophils also increased at all stages of the experiment (both on the 2nd and 4th week of sodium glutamate administration). At the same time, we found no significant difference between the obtained indicators in animals of the I and II groups.

So, against the background of sodium glutamate administration at a dose of 15 mg/kg of body weight, changes in their cellular composition occur in lymphoid nodes and pre-nodules, as well as in diffuse lymphoid tissue of GMM compared to rats of the control group. As early as on the 2nd week of the experiment, the number of small lymphocytes in these lymphoid structures of the GMM in rats increases compared to the indicators in control animals, and 4 weeks after the administration of monosodium glutamate, the density of small lymphocytes increases even more in all lymphoid structures of the GMM.

DISCUSSION

The Various experimental and clinical studies are being conducted on the effects of monosodium glutamate on the organs and systems of the body. The results of a clinical study conducted by Boutry C. et al. with the involvement of volunteers who took monosodium glutamate in a dose of 2 g within 7 days indicate an increase in the volume of the portal cavity of the stomach in comparison with such data of the control group. The authors attribute the obtained result to an increase in gastric secretion, while the rate of gastric emptying remained unchanged [5].

The results of Xu JZ et al (2022) showed that administration of 30 mg/kg monosodium glutamate had no significant effect on serum C-reactive protein, trimethylamine N-oxide, angiotensin II, intestinal interleukin (IL)-1 β , IL-6, factor tumor necrosis- α , secretory IgA, and fecal albumin in mice, but promotes intestinal inflammation and changes in intestinal flora. Moreover, the administration of 1500 mg/kg of monosodium glutamate increased the risk of cardiovascular diseases and damaged the intestinal structure and disturbed the composition of the intestinal microflora. The authors also established that with an increase in the dose of monosodium glutamate, inflammatory changes in the intestines of mice increase, while intestinal immunity sharply decreases [6].

The results of our research also indicate the reaction of the immunocompetent tissue of the digestive tract to

the administration of monosodium glutamate to rats at a dose of 15 mg/kg of body weight. The activity of immune processes when sodium glutamate is administered is indicated by an increase in the number of macrophages in all lymphoid formations of GMM in an experiment in rats. Changes in all cell types of elements of lymphoid nodules, pre-nodules and diffuse lymphoid tissue of GMM were also found, which indicates the reaction of the body, including the local immunity of the stomach, to sodium glutamate intake. This is manifested by an increase in the density of cellular elements of the lymphoid tissue of the GMM and indicates a protective reaction of the animal body to the action of a negative factor, namely, the food additive – monosodium glutamate.

CONCLUSIONS

Administering monosodium glutamate to rats at a dose of 15 mg/kg of body weight for 2 weeks leads to an increase in the density and size of lymphoid structures of the mucous membrane in all parts of the stomach with a predominant increase in the number of immunocompetent cells between the bottom of the gastric glands and the muscle plate. At the same time, more pronounced changes were found in the number of small lymphocytes, which tend to decrease by the 2nd week of the experiment, and vice versa – their density increases by the 4th week of monosodium glutamate administration.

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Conflict of interest:

The Authors declare no conflict of interest.

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