HISTOLOGICAL AND MORPHOLOGICAL CHANGES OF THE VASCULAR BED OF THE THYMUS IN WHITE RATS UNDER THE INFLUENCE OF MONOSODIUM GLUTAMATE

DOI: 10.36740/WLek202311124

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ABSTRACT

The aim: To evaluate the effect of 28-day oral administration of MSG at the rate of 30 mg/kg of body weight on histological and morphometric parameters of the vascular bed of the thymus in rats.

Materials and methods: The scientific experiment was conducted on 20 white non-linear rats of reproductive age (4-5 months) weighing from 220 to 280 g, which were divided into two groups (10 rats each). Depending on the term of decapitation, the experimental animals were divided into two groups (10 rats in each group). We studied the effect of 14 and 28 days of MSG administration on the body of rats (I and II groups of experimental rats). The experimental animals were daily orally treated with MSG at a dose of 30 mg/kg body weight, which was dissolved in 0.5 ml of dechlorinated tap water at room temperature. Control rats of III and IV groups (5 rats in each of the control groups) were injected with a placebo (0.5 ml of dechlorinated tap water at room temperature) for 14 and 28 days. Intact animals of III and IV groups were also decapitated on the 14th and 28th days of the experiment, respectively.

After the end of the experiment, animals were decapitated under light ether anesthesia. After decapitation, the animals were dissected into the chest cavity to remove the thymus. Histological preparations were studied using a MICROmed SEO SCAN light microscope and a Vision CCD Camera. Morphometric studies were carried out using VideoTest-5.0, KAARA Image Base and Microsoft Excel programs on a personal computer.

Results: During the microscopic examination of histological preparations of the retrosternal gland in experimental animals of the 1st group (daily administration of MSG at the rate of 30 mg/kg of body weight for 14 days), it was established that the lumen of the arteries is moderately filled with blood elements. The veins are dilated with a changed shape and filled with blood. The following ultrastructural changes were detected in the experimental animals of group I: the lumen of arteries, arterioles and venules is slightly expanded, the nuclei of endotheliocytes are enlarged, occupy a significant part of the cytoplasm, the karyolem forms intussusceptions. The plasmolemma of the lumenal surface of endotheliocytes forms numerous microvilli. At the same time, organelles in the cytoplasm of endotheliocytes lose their contours. After 28 days of exposure to MSG at a dose of 30 mg/kg of body weight in rats (II group of experimental animals), structural changes in the vascular bed of the thymus worsened. The wall of arteries and arterioles is more thickened and swollen, collagen fibers are stratified. In their lumen, there are many uniform elements attached to the vascular wall and testify to thrombus formation. Perivascular edema is determined. The diameter of hemocapillaries is increased, their basal membrane is swollen. Veins and venules are also dilated, full blood, interendothelial contacts in the vessel wall are dilated, the basement membrane is damaged. This contributes to the diapedesis of blood plasma through the vessel wall, which leads to perivascular edema. Conclusions: Administration of MGS to rats at a dose of 30 mg/kg of body weight for 14 days leads to violations of the morphometric indicators of the vascular bed in the thymus, namely, to an increase in the outer and inner diameter of the arteries, an increase in the area of the middle membrane and the lumen of the vessels, which tend to progress with maximum indicators on the 28th day of the experiment. 2. The study of the vascular bed of the thymus against the background of taking MSG in a dose of 30 mg/kg of the weight of rats indicates the most pronounced changes in hemocapillaries, mainly on the 28th day of the experiment, which is manifested by an increase in their outer diameter. In the lumen of the hemocapillaries, deformed erythrocytes are identified, arranged in the type of "coin columns".

KEY WORDS: monosodium glutamate, thymus, vessels

Wiad Lek. 2023;76(11):2491-2496

INTRODUCTION

The flavor additive of monosodium glutamate (MSG) is widely used in the industry for the production of food products, medicines, fodder and feed additives for industrial livestock production of whey [1]. 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. animals, in particular rodents [2].

The effect of monosodium glutamate on the body of animals and humans manifests itself mainly in the form of metabolic disorders. Increasing the daily dose of MSG even by 1 g. significantly increased the risk of developing metabolic syndrome and obesity in people, regardless of lifestyle (diet, physical activity). Studies on laboratory animals have shown an increase in body weight, the development of insulin resistance and other hormonal disorders, changes in serum biochemical indicators [1, 2].

Monosodium glutamate has a complex mechanism of action, which can be conditionally divided into indirect and direct. The indirect effect of MSG on the body is realized by affecting the neuroendocrine system and damage to the hypothalamus. As a result of damage to the autonomic nervous system, the work of controlled organs and systems is disrupted. The direct effect of MSG is the occurrence of inflammatory processes in tissues, infiltration by lymphoid cells, edema and microcirculation disorders. As a result, hypoxia and fibrosis develop in tissues. Damage to the endothelium of vessels causes disruption of histo-hematic barrier functions and hemorrhage [3].

The the study of the influence of MSG on changes in the vascular bed in various organs and systems is an urgent task of modern medical science.

THE AIM

The aim is to evaluate the effect of 28-day oral administration of MSG at the rate of 30 mg/kg of body weight on histological and morphometric parameters of the vascular bed of the thymus in rats.

MATERIALS AND METHODS

The scientific experiment was conducted on 20 white non-linear rats of reproductive age (4-5 months) weighing from 220 to 280 g, which were divided into two groups (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). Experimental and control animals were housed in separate boxes in the vivarium. All experimental animals were kept in the conditions of the vivarium of the Lviv National Medical University named after Danylo Halytskyi.

Depending on the term of decapitation, the experimental animals were divided into two groups (10 rats in each group). We studied the effect of 14 and 28 days of MSG administration on the body of rats (I and II groups of experimental rats). The experimental animals were daily orally treated with MSG at a dose of 30 mg/kg body weight, which was dissolved in 0.5 ml of dechlorinated tap water at room temperature. Control rats of III and IV groups (5 rats in each of the control groups) were injected with a placebo (0.5 ml of dechlorinated tap water at room temperature) for 14 and 28 days. Intact animals of III and IV groups were also decapitated on the 14th and 28th days of the experiment, respectively.

After the end of the experiment, animals were decapitated under light ether anesthesia, with the animals previously weighed. After decapitation, the animals were dissected into the chest cavity to remove the thymus. After dissection of the ventral wall of the chest cavity and the front surface of the neck, the mammary gland was removed. The obtained material was processed according to generally accepted methods. For histological examination, tissue blocks of the thymus were fixed in a 10.0% solution of neutral formaldehyde. After fixation, the material was washed, dehydrated in a series of alcohols of increasing concentration, passed through chloroform and poured into paraplast. Sections of tissue with a thickness of 5-7 µm were prepared on a rotary microtome, placed on glass, stained with hematoxylin-eosin according to the generally accepted method. Histological preparations were studied using a MICROmed SEO SCAN light microscope and a Vision CCD Camera. Morphometric studies were carried out using VideoTest-5.0, KAARA Image Base and Microsoft Excel programs on a personal computer. The research was carried out in the specified terms of the experiment in preparations stained with hematoxylin and eosin. The relative area of the cortical substance of the thymus lobe, the relative area of the medullary substance of the thymus lobe, the cortical-cerebral index, the thickness of the capsule, the number of thymocytes per unit area in the cortical and medullary substances of the thymus lobe, as well as the diameter of small thymocytes in the compositions of the cortical and medullary substance of the thymus lobe were studied morphometrically.

In accordance with the purpose of the scientific work, the arteries of the muscular type of small and medium caliber were studied. The values of the external (D_1) and internal (D_2) diameters, the area of the middle membrane (media) (Sm) and the area of the lumen (SI) were determined. The assessment of the functional state of the blood vessels was carried out by calculating the Voogenvoort arterial permeability coefficient (VC), which is the ratio of the wall area to the area of the lumen - VC= (Sm/SI) x 100%.

The outer diameter (D) of the blood capillaries of the cortical and medullary substances of the thymus lobes

was also measured.

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

The parameters of the arteries, that supply blood to the thymus in rats were studied (interlobular and intralobular arteries of the retrosternal gland, from which the arcuate arteries that form the vessels of the hemomicrocirculatory bed of the organ branch out).

During the microscopic examination of histological preparations of the retrosternal gland in experimental animals of the 1st group (daily administration of MSG at the rate of 30 mg/kg of body weight for 14 days), it was established that the lumen of the arteries is moderately filled with blood elements. The veins are dilated with a changed shape and filled with blood. The following ultrastructural changes were detected in the experimental animals of group I: the lumen of arteries, arterioles and venules is slightly expanded, the nuclei of endotheliocytes are enlarged, occupy a significant part of the cytoplasm, the karyolem forms intussusceptions. The plasmolemma of the lumenal surface of endotheliocytes forms numerous microvilli. At the same time, organelles in the cytoplasm of endotheliocytes lose their contours.

During the microscopic examination of the histological preparations of the first group of animals, an increase in the lumen of the veins, which are filled with blood-forming elements, was established. The general increase in the diameter of the vessels of the microcirculatory bed is determined. At the same time, erythrocytes in the lumens of hemocapillaries are arranged in the type of "coin columns". Single erythrocytes are detected in the intercellular space, which indicates damage to the vascular wall. The lumen of the capillaries is slightly narrowed, which occurs as a result of the swelling of the endotheliocytes and the protrusion of the plasmolemma into the lumen of the vessels. The lumen of arterioles is also slightly expanded. The basement membrane of the venules is thickened in some places.

After 28 days of exposure to MSG at a dose of 30 mg/kg of body weight in rats (II group of experimental animals), structural changes in the vascular bed of the thymus worsened. The wall of arteries and arterioles is more thickened and swollen, collagen fibers are stratified. In their lumen, there are many uniform elements attached to the vascular wall and testify to thrombus formation. Perivascular edema is determined. The

diameter of hemocapillaries is increased, their basal membrane is swollen. Veins and venules are also dilated, full blood, interendothelial contacts in the vessel wall are dilated, the basement membrane is damaged. This contributes to the diapedesis of blood plasma through the vessel wall, which leads to perivascular edema.

The release of formed blood elements into the parenchyma is also determined. In the lumen of the hemocapillaries, erythrocytes are deformed, also arranged in a "coin column" type, as in animals of the 1st group. Erythrocytes often attach to the lumenal surface of endotheliocytes. The wall of arteries and arterioles is thickened due to swelling of endotheliocytes. Initial signs of sclerosis in arteries and arterioles are determined.

The most pronounced microscopic changes in animals of the II group were found in the blood capillaries, namely, an increase in their outer diameter, the basal membrane of hemocapillaries was significantly thickened and stratified. In the lumen of hemocapillaries, deformed clusters of erythrocytes are determined. In animals of the II group, electron microscopy revealed enlarged nuclei of endotheliocytes of an irregular elongated shape with protrusions and depressions in the wall of hemocapillaries. The cytoplasm of endotheliocytes is thinned, enlightened. The lumen of the hemocapillaries is reduced due to the fact that the plasmalemma. of the lumenal surface of endotheliocytes forms numerous protrusions and microvilli in the hemocapillary lumen.

The parameters of the arteries supplying blood to the thymus in rats were also assessed by the morphometric method. Their outer diameter was determined, which is 50.12±1.12 µm in animals of III and 49.95±1.07 µm in rats of IV groups, the inner diameter is 25.70±1.02 µm in III and 25, 63±1.14 µm in animals of the IV group, the area of the middle membrane (1428.23±11.46 µm2 in animals of the III and 1441.25±10.74 µm2 in rats of the IV group), the area of the lumen (513.56 \pm 5, 37 μ m2 in animals of III and 514.02±4.06 µm2 in rats of IV groups), the Vogenvoort coefficient, which was 290.67±3.15% in animals of III group and 289.77±5.56% in rats of IV group. The indicator of the outer diameter of the blood capillaries in the thymus lobules was as follows in the groups of control animals: in the cortical substance -12.07±0.55 µm in animals of III and 12.23±0.61 µm in rats of IV group; in the brain substance, 24.06±1.07 µm in III animals and 24.21±1.18 µm in IV group rats (Table I). As indicated by the results of the statistical analysis, we did not establish a significant difference between the indicators in rats of III and IV groups. Accordingly, their indicators were taken as the norm, with which the obtained results of the I and II groups of experimental rats were subsequently compared.

	Groups of studied animals			
Indicators	Experimental groups		Control groups	
	I group (n=10)	li group (n=10)	III group (n=5)	IV group (n=5)
D ₁ arteries, μm	53,04±1,05*	54,88±1,14*	50,12±1,12	49,95±1,07
D ₂ , μm	26,87±1,12*+	22,04±0,86*	25,70±1,02	25,63±1,14
Sm arteries, μm ²	1603,21±12,55**	1785,35±14,78**++	1428,23±11,46	1441,25±10,74
SI arteries, µm ²	560,23±5,48*+	535,64±4,12*	513,56±5,37	514,02±4,06
VC, %	289,12±3,26*	328,77±3,08*+	290,67±3,15	289,77±5,56
D hemocapillaries of the cortical substance, μm	16,02±0,71*	18,95±0,46**+	12,07±0,55	12,23±0,61
D hemocapillaries of the brain substance, µm	29,88±0,27*	34,12±0,45**+	24,06±1,07	24,21±1,18

Table I. Morphometric indicators of the vascular bed of the retrosternal gland in experimental rats

Note: the difference between the indicators of rats of experimental groups (I and II groups) and control groups (III-IV groups) 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; + + - p < 0.01.

The analysis of the results of the morphometric parameters of the vascular bed of the thymus in rats of the I group (after 14 days of taking MSG) indicates a significant increase in the indicator of the outer diameter of the arteries (up to $53.04 \pm 1.05 \mu m - p < 0.05$). The maximum pronounced increase of this indicator was established on the 28th day of the experiment in rats of the II group (increase to 54.88±1.14 µm). In rats of both experimental groups, the external diameter of arteries was significantly different from this indicator of intact rats of III and IV groups - p<0.05. On the contrary, the internal diameter of the arteries was maximally increased in rats of the I group and significantly differed from this indicator in the rats of the II group (p<0.05). In rats of the II group, the inner diameter of the arteries was lower than this indicator of the control animals, namely - by 3.66±0.16 µm from this indicator in the rats of the III group and by 3.59±0.28 µm from the indicator in the animals of the IV group - p<0.05.

A significant increase in the area of the middle membrane and the area of the lumen of the arteries in the retrosternal gland was established in rats of both experimental groups in comparison with such indicators in the animals of the control groups. At the same time, if the maximum pronounced deviation from the norm in the analysis of the area of the middle layer of arteries was found in animals of the III group (its increase to 1785.35±14.78 µm2 - p<0.01), then on the contrary - the area of the lumen of the arteries increased maximally in animals of I group (560.23±5.48 µm2 - p<0.05), in comparison with the data of control animals. If on the 14th day of the experiment, the VC in the animals of the I group actually did not differ from that in the rats of the control groups, then on the 28th day of receiving MSG, it was found to have significantly increased in comparison with the animals of the control groups (up to 328.77±3.08 % - p<0.05).

During the morphometric study of histological preparations, a reliable gradual increase in the outer diameter of hemocapillaries was established in both the cortical and medullary substances of the thymus with the maximum values in rats of the III group – up to $18.95\pm0.46 \mu m$ in the cortical substance of the lobules and up to $34.12\pm0.45 \mu m$ in the brain substance of the lobules, which, accordingly, significantly exceed these indicators of intact animals (p<0.01).

So, already 14 days after the introduction of MSG to rats at a dose of 30 mg/kg of body weight, changes in the vascular bed of the thymus in experimental animals are determined, which have a tendency to progress with maximally pronounced changes on the 28th day of the experiment.

DISCUSSION

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 "flavor enhancer" for more than 100 years, is the fifth type of taste in Japan - "umami", after sour, bitter, salty and sweet. It was first discovered by a Japanese scientist, Professor Kikunae Ikeda and introduced into mass production as one of the most common food additives after salt and pepper. In the professional literature, information about the negative impact of MSG on the structural organization of organs, in particular the nervous, digestive, immune and other systems, is increasingly appearing [4].

Experimental studies indicate that in glutamate-induced obesity, excess fat accumulates in adipose tissue as a result of increased cholesterol levels, which leads to cardiovascular pathology [5]. The preliminary data obtained by us also indicate a violation of lipid metabolism in rats when MSG is administered at a dose of 30 mg/kg of body weight against the background of structural changes in organs, including the thymus. In an experimental study by Aghajani M. et al. (2017), it was established that the introduction of MSG in combination with a high-calorie diet led to oxidative stress due to increased levels of nitric oxide. This, in turn, increases the area of damage during myocardial infarction in experimental animals [6].

The data obtained by us also confirm the negative effect of MSG on the vascular structures of the thymus in rats receiving the dietary supplement at a dose of 30 mg/kg body weight. Already on the 14th day of the experiment, the expansion of the lumen of the arteries and arterioles of the thymus was established, which indicates vasodilatation. The lumen of the veins and venules is increased, they are full of blood. Two-week administration of GN contributes to the narrowing of the lumen of the hemocapillaries of the thymus in rats.

Destructive changes in the vascular bed of the thymus after 28 days of taking GN in a dose of 30 mg/ kg body weight of rats deepen even more. Veins and venules are more dilated, full-blooded. The basement membrane is swollen. The wall of arteries and arterioles are thickened, swollen, there are many shaped elements in their lumen, which is a sign of thrombus formation. Perivascular edema increases. Hemocapillaries increase in diameter, both in the cortical and medullary substances of the thymus, their basal membrane is significantly thickened.

Therefore, as indicated by the results of our experimental study, structural changes in the vascular bed of the thymus occur against the background of taking MSG in rats. This is manifested by a change in the lumen of blood vessels, vasodilatation, as well as sclerotic changes, which tend to progress depending on the term of appointment of this dietary supplement.

CONCLUSIONS

Administration of MGS to rats at a dose of 30 mg/kg of body weight for 14 days leads to violations of the morphometric indicators of the vascular bed in the thymus, namely, to an increase in the outer and inner diameter of the arteries, an increase in the area of the middle membrane and the lumen of the vessels, which tend to progress with maximum indicators on the 28th day of the experiment.

2. The study of the vascular bed of the thymus against the background of taking MSG in a dose of 30 mg/kg of the weight of rats indicates the most pronounced changes in hemocapillaries, mainly on the 28th day of the experiment, which is manifested by an increase in their outer diameter. In the lumen of the hemocapillaries, deformed erythrocytes are identified, arranged in the type of "coin columns".

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The study was performed within the framework of the scientific topics "Polymorbid Pathology of Digestive System Diseases, Features of Pathogenesis and the Possibility of Correction" (state registration number 0118U004365) researched by the Department of Propedeutics of Internal Diseases of State University "Uzhhorod National University" and "Clinical and Pathogenetic Features of Polymorbid Diseases in the Digestive System and Development of Differentiated Therapy Scheme in the Conditions of the COVID-19 Pandemic" (state registration number 0121U110177).

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Conflict of interest:

The Authors declare no conflict of interest.

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Received: 14.05.2023 **Accepted:** 22.10.2023

A - Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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