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REMODELING OF THE GASTRIC FUNDIC VASCULATURE UNDER THE EFFECT OF COMPLEX OF MONOSODIUM GLUTAMATE, SODIUM NITRITE AND PONCEAU 4R

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The paper presents the data of morphometric study of the effect of the food additives complex on the state of the vasculature of the stomach fundus under the action of the complex of monosodium glutamate, sodium nitrite and Ponceau 4R food additives. It was found that in the early stages of the experiment there was spasm of blood vessels of the hemomicrocirculatory bed due to the direct effect of food additives on the mucous membrane, which led to increased diameters of submucosal vessels as a result of hemodynamic conditions of the mucous membrane. Further development of the inflammatory reaction and the phenomenon of hypoxia led to compensatory-restorative reactions, but complete recovery did not occur, which at the end of the experiment was expressed by decompensation of the resistive link, metabolic spasm and increased lumen of the capacitive link. **Key words:** fundus of stomach, vessels, food supplements, mucous membrane, rats.

А.І. Ячмінь, Г.А. Єрошенко, К.В. Шевченко, С.М. Білаш, О.Д. Лисаченко, В.М. Соколенко, Н.М. Шарлай, О.В. Клепець РЕМОДЕЛЮВАННЯ СУДИННОГО РУСЛА ФУНДАЛЬНОГО ВІДДІЛУ ШЛУНКА ПРИ ДІЇ КОМПЛЕКСУ ГЛУТАМАТУ НАТРІЮ, НІТРИТУ НАТРІЮ ТА ПОНСО 4R

В роботі наведені дані морфометричного дослідження дії комплексу харчових добавок на стан судинного русла фундального відділу шлунка при дії комплексу харчових добавок глутамату натрію, нітриту натрію та Понсо 4R. Було встановлено, що на ранніх етапах експерименту спостерігався спазм судин гемомікроциркуляторного русла внаслідок безпосереднього впливу складових харчових добавок на слизову оболонку, що призвело до збільшенням діаметрів судин підслизової основи, як результат порушення гемодинамічних умов у слизовій оболонці. В подальшому розвиток запальної реакції та явища гіпоксії призвело до виникнення компесаторно-відновлювальних реакцій, але повного відновлення не відбувалось, що на кінець експерименту проявлялось декомпенсацією резистивної ланки, спазмом обмінної та збільшенням просвіту ємнісної ланки.

Ключові слова: фундальний відділ шлунка, судини, харчові добавки, слизова оболонка, щури.

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Currently, the aggressive impact of food products and various chemical agents on the gastrointestinal mucosa caused the increase in chronic diseases of the digestive system, and the stomach is one of the organs of the digestive system, which is first affected by various critical conditions [2, 6].

The proper functioning of organs and systems depends on the state of the sections of the microcirculation, as well as the state of larger vessels that provide blood inflow and outflow from the organs.

Due to increasing need for food in modern society and to increase demand, manufacturers use various types of food additives in the production of food. Nowadays, synthetic food additives are considered the most dangerous, as they are xenobiotics that the human body has not experienced during its evolutionary development and, therefore, it lacks enzymes that can convert them into non-toxic metabolites [8].

The analysis of the content of food additives in domestic and foreign products showed that the most common substances used in production are monosodium glutamate, sodium nitrite and synthetic dye Ponceau 4R.

Monosodium glutamate (E621) is currently widely used in the marketing network to enhance the flavor. The addition of monosodium glutamate to food products (up to 10 g/kg) enhances their natural taste properties, weakened during processing and storage and disguises certain negative components of taste and smell. Today, about 50 % of store products are supplemented with this additive, while the average daily human consumption in European industrialized countries is about 0.3–1.0 g [3]. Although food safety regulators consider the consumption of monosodium glutamate to be safe, some preclinical and clinical studies have questioned its safety, especially after chronic exposure. The controversy is probably due to the fact that endogenous glutamate plays a role in both physiological and pathological processes [15].

Sodium nitrite is widely used as a food additive in the production of meat products, as well as canned fish to improve the consumer properties of the product and increase the stability of the product

during storage. The nitrites, used as a dietary supplement, not only changes the composition and function of hemoglobin, but also reduces the functional activity of mitochondria, which leads to a deficiency in the tissues of macroenergetic compounds [8, 12].

The dramatic increase and uncontrolled use of synthetic food colorants [4], added to various types of goods to enhance their appearance or compensate for natural color variations is of great concern [9]. Ponceau 4R (E 124) is a sulfonated monoazo dye made by combining diazotized naphthionic acid with G acid and converting the coupling product into the trisodium salt, which is manufactured in the form of water-soluble red powder or granules [5, 8]. Ponceau 4R has been reported to increase the production of F2-isoprostanes from blood neutrophils at all concentrations studied, thus provoking a high ability to induce proinflammatory reactions *in vitro*, indicating a potential health risk [7, 14].

Therefore, the study of the effects of various food additives on humans and animals is one of the most topical issues to date. Such studies are extremely important for the development of a scientifically grounded strategy to increase human tolerance to xenobiotics by activating genetically engineered mechanisms, as well as by creating new perfect adaptogenes.

The purpose of the study was to establish the dynamics of changes in the metric parameters of the hemomicrocirculatory bed of the mucosa and vessels of the submucosal plate of the fundus of the rat stomach in norm and under the action of a complex of glutamate sodium, sodium nitrite and Ponceau 4R food additives.

Material and Methods. Outbred mature male rats (n=84) were involved into the experiment. The rats of control group consumed drinking water and received 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 evaluation of the rats' adaptive behavior with the use of the "open field" test was made prior the animals' sacrifice with the subsequent processing of the results by the methods of variation statistics using the *Excel* software [1].

The rats were sacrificed within 1, 4, 8, 12 and 16 weeks under thiopentone anesthesia overdose. Upon euthanasia, one part of the fragments of the gastric fundic wall was fixed in 10% neutral formalin solution for three days and another one in 2.5 % glutaraldehyde solution. Subsequently, the pieces of the gastric wall, fixed in formalin, were embedded into paraffin and Epon [10]. The sections were obtained using the sliding microtome and mounted on the slides. The sections were stained with hematoxylin and eosin and polychrome stain, enclosed into polystyrene and studied in the light microscope. The light microscope equipped with DCM 900 digital microphoto attachment and software, adapted to the studies, have been used for microimaging and morphometric study. Statistical processing of the morphometric data was made using the *Excel* software [1].

Results of the study and their discussion. Morphometric study of the microvasculature of the rat gastric fundus showed that in control rats, the diameter of the lumen of arterioles, capillaries and venules was $16.28\pm0.18 \mu m$, $6.39\pm0.04 \mu m$ and $21.41\pm0.25 \mu m$, respectively (table 1).

Table 1

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Parameters	Mucous membrane			
	Arterioles	Capillaries	Venules	
Control	16.28±0.18	6.39±0,04	21.41±0,25	
Week 1	16.01±0.05 *	5.94±0.03 *	20.38±0,14 *	
Week 4	16.71±0.23 *.**	7.42±0.03 *.**	20.98±0.13 * **	
Week 8	16.09±0,05 *.**	5.41±0.02 *.**	24.70±0.23 *.**	
Week 12	17.76±0,22 *.**	5.71±0.03 * **	21.93±0.19 *.**	
Week 16	18.20±0.19 * **	6.04±0.02 * **	25.45±0.33	

Morphometric characteristics of the elements of the blood microcirculatory bed of the rat gastric fundic mucosa, µm

Notes: * - p < 0.05 compared to the control group; p<0.05 compared to the previous time period of the observation.

The capillaries had a typical location in the loose fibrous connective tissue of the lamina propria in the spaces between the glands of the gastric fundus. The inner layer of the capillary wall was formed by the endothelium, which provides transcapillary transport, the cells of which were located on the basement

membrane with the low- basophilic flattened nuclei. Pericytes with ovoid nuclei were detected in the basement membrane fissure. Erythrocytes, arranged in one row, were visualized in the lumen (fig. 1).



Fig. 1. Capillaries in the lamina propria of the gastric fundus of control rats. Polychrome stain. Ocular lens: 10×magnification, objective lens: 100×magnification.

Within 1 week of consumption of complex of food additives the resistance vessels responded with a decrease in the vascular lumen diameter by 1.66 %, accounting for $16.01\pm0.05 \mu$ m; the diameter of the lumen of the exchange vessels was by 7.04 % smaller compared to the values of the control group and accounted for $5.94\pm0.03 \mu$ m (p<0.05). The capacitance vessels also responded by the lowering of the morphometric parameters by 4.84 % with its mean values of $20.38\pm0.14 \mu$ m (p<0.05).

The histological study made at the early time of observation revealed spastic phenomena in the arterioles of the mucous membrane; the nuclei of the endothelial cells protruded in the lumen, and the vast majority of nuclei of the smooth myocytes were

orbicular in the tunica media of the arterioles. No blood corpuscles were detected in the lumens. A small number of lymphoid cells were found in the perivascular loose connective tissue (fig. 2a). The capacitance vessels were folded, with a thinned wall and signs of desolation in the lumens of the venules (fig. 2b).



Fig. 2a. Spastic phenomena in the arterioles of the mucous membrane on week 1 of the experiment. H&E stain. Ocular lens: 10×magnification, objective lens: 40×magnification.



Fig. 2b. Folded venule of the rat gastric fundic mucosa on week 1 of the observation. H&E stain. Ocular lens: 10×magnification, objective lens: 40×magnification.

b

On week 4 of the experiment, the combined effect of monosodium glutamate, sodium nitrite and Ponceau 4R led to increase in the mean values of the diameter of the lumen of the arterioles (16.71±0.23 μ m), which was by 4.37 % greater than the values of the previous period of the experiment and by 2.64 % significantly greater than the values of the control group (p <0.05). Morphometric parameters of the diameter of the capillary lumen were by 24.92 % and 16.12 % significantly higher than the value on the week 1 of the experiment and the value in the control group of rats, respectively, and accounted for 7.42±0.03 μ m (p<0.05). The mean values of the diameter of the lumen of the venules were 20.98±0.13 μ m, which was by 2.94 % significantly greater than the values of the previous time period of the experiment, though was by 2.01 % significantly lower than the control values (p<0.05).

The exchange vessels responded by dilatation, caused by the development of tissue hypoxia in the gastric wall due to spasm of resistance vessels at the early time periods of the experiment. The capillary wall was thinned. The luminal contour of endotheliocytes was uneven. The basement membrane preserved continuity. Erythrocytes and a large amount of blood plasma of low optical density were noted in the lumens of the vessels (fig. 3).



Fig. 3. Capillaries of the lamina propria of the rat gastric fundic mucosa on week 4 of the experiment. Polychrome stain. Ocular lens: $10 \times magnification$, objective lens: $100 \times magnification$.

On week 8 of consumption of complex food additives, the mean values of the diameter of the arteriole lumen was 3.71 % and 7 % significantly lower than the values of week 4 of the experiment and the values of the control group of animals, respectively, accounting for $16.09 \pm 0.05 \,\mu m \,(p < 0.05)$. The values of the diameter of the lumen of the capillaries also showed a tendency to decrease, and was equal to 5.41±0.02 µm, which was by 27.09 % significantly lower than the values of the previous time of experiment and by 15.34 % lower than the control values (p < 0.05). The diameter of the lumen of the venules was 24.70±0.23 µm, which was by 17.73 % significantly greater than the values of the previous time period of observation, and by 15.37 % greater than the values in the control group of rats (p < 0.05).

Histological study revealed the phenomena of arteriole spasm. Endothelial cell nuclei protruded in the lumen of the vessels, the tunica intima was not visualized. No blood corpuscles were noted in the lumens. The wall was thickened and wavy. Leukocytes were detected perivascularly (fig. 4a). In the venules of the gastric fundus, dilatation with disrupted blood perfusion was detected, which was manifested by the plethora in the lumens of the capacitance microvessels; the lumens were densely filled with blood corpuscles. The venule wall retained the organ structure, though was thinned (fig. 4b).



Fig. 4a. Spasm of the arterioles of the mucous membrane on week 8 of the observation. H&E stain. Ocular lens: 10×magnification, objective lens: 40×magnification.



Fig. 4b. Plethora of the capacitance vessels on week 8 of consumption of complex of monosodium glutamate, sodium nitrite and Ponceau 4R. Polychrome stain. Ocular lens: 10×magnification, objective lens: 40×magnification.

On week 12 of consumption of monosodium glutamate, sodium nitrite and Ponceau 4R food additives the mean values of the diameter of the lumen of the resistance vessels was $17.76\pm0.22 \mu m$, that was by 10.38 % and 9.09 % significantly greater than the values on week 8 of the experiment and the values

Table 2

of the control group of animals, respectively (p<0.05). The mean values of the diameter of the lumen of the exchange vessels were by 5.55 % greater than the values of the previous time period of the experiment and accounted for 5,71±0,03 μ m, though were by 10.64 % significantly lower than the control values (p<0.05). The diameter of the venule lumen was 21.93±0.19 μ m, that was by 11.21 % significantly lower than the values of the previous time period, though was by 2.43 % significantly greater than the values of the control group of animals (p<0.05).

On week 16 of the consumption of complex of food additives led to increase in the mean values of the diameter of the arteriole lumen by 2.48 %. compared to the values of the previous time period of the experiment. accounting for 18.20 \pm 0.19 µm. and was by 11.79 % significantly greater than the values of the control group of animals (p<0.05). The mean values of the diameter of the capillary lumen was 6.04 \pm 0.02 µm. that was by 5.55 % significantly greater than the values on the week 12 of the experiment. though was by 5.48 % lower than the values of the control group of animals (p<0.05). The diameter of the lumen of the venules of the mucous membrane decreased by 11.21 % and accounted for 25.45 \pm 0.33 µm at the end of the experiment. though was by 2.43 % significantly greater than the values of the control group (p<0.05).

The morphometric study of the vessels of the submucosal layer has established that the diameter of the lumen of the arteries and veins was $36.42\pm3.38 \mu m$ and $48.94\pm4.02 \mu m$. respectively (table 2).

Submucosal layer			
Parameters	Arteries	Veins	
Control	36.42±0.38	48.94±4.02	
Week 1	45.35±2.94 *	65.44±2.03 *	
Week 4	47.83±1.02 *	60.44±2.12 *.**	
Week 8	45.08±3.17 *	54.22±3.14 *.**	
Week 12	42.36±2.84 *	60.73±2.71 *.**	
Week 16	35.36±0.02 *.**	67.43±3.18 *.**	

Morphometric characteristics of the vessels of the submucosal layer of the rat gastric fundus, µm

Notes: * - p < 0.05 compared to the control group; p<0.05 compared to the previous time period of the observation.

Within 1 week of the consumption of monosodium glutamate. sodium nitrite and Ponceau 4R the diameter of the lumen of the arteries of the submucosal layer increased by 24.52 % and accounted for $45.35\pm2.94 \mu m$; a significant increase in the mean values of the morphometric parameters of the diameter of the lumen of the veins by 33.71 % was also noted that accounted for $65.44\pm2.03 \mu m$ (p<0.05).

On week 4 of the experiment. insignificant dilatation of the lumen of the arteries with inner diameter of $47.83\pm1.02 \mu m$ was noted; however. the above values were still significantly greater than the values of the control group by 31.33 % (p<0.05). The diameter of the lumen of the veins was by 7.64 % significantly lower than the values on week 1 of the experiment. accounting for $60.44\pm2.12 \mu m$. though it was still by 23.50 % significantly greater than the values of the control group of rats (p<0.05).

Starting from week 4 of the observation the lumens of the arteries of the rat gastric submucosal layer was dilated. The wall retained a layered structure. The nuclei of endothelial cells were elongated and oriented parallel to the basement membrane. Externally. 3 layers of smooth myocytes and loose fibrous connective tissue of the adventitial membrane with single fibroblast bodies were detected. Blood corpuscles were found in the lumens. The surrounding connective tissue showed morphological signs of hyperhydration.

On week 8 of consumption of complex of food additives the diameter of the arteries of the submucosal layer of the gastric fundus was $45.08\pm3.17 \mu m$. that was insignificantly greater than the values of the previous time period of the experiment. though it was by 23.78 % significantly greater the control values (p<0.05). The diameter of the lumen of the capacitance vessels was by 10.29 % significantly greater than the values of the previous time period of the experiment. accounting for $54.22\pm3.14 \mu m$ and was by 10.79 % significantly greater than the values of the control group (p<0.05).

On week 12 of the experiment, the combined effect of the food additives led to insignificant decrease in the inner diameter of the arteries of the submucosal layer, accounting for 42.36 ± 2.84 µm, though it was by 16.31 % significantly greater than the control values (p<0.05). The veins of the

submucosal layer responded by the significant (by 12.01 %) dilatation of the luminal diameter compared to the values of the previous time period of the experiment. accounting for $60.73\pm2.71 \mu m$. and was by 24.09 % greater the control values (p<0.05).

The study findings of the effect of monosodium glutamate, sodium nitrite and Ponceau 4R on week 16 complex food additives have established that the diameter of the arteries' lumen of the submucosal layer of the rat gastric fundus was $35.36\pm0.02 \ \mu\text{m}$. that was by $16.53 \ \%$ significantly lower than the values on week 12 of the experiment and by 2.91 % lower than the values of control group animals (p<0.05). The diameter of the veins' lumen significantly increased by $11.03 \ \%$ compared to the findings of the previous time period of the experiment accounting for $67.43\pm3.18 \ \mu\text{m}$. and was by $37.78 \ \%$ significantly greater than the values of control group (p<0.05).

Thus, the effect of the monosodium glutamate, sodium nitrite and Ponceau 4R complex food additives at the early stages of the experiment led to the decrease in the diameter of the vessels of the microcirculatory bed of the gastric fundic mucosa, which is apparently associated with the direct impact of the food additives compounds on the mucous membranes [10], and the response of the vascular bed to the action of various exogenous factors [11]. Larger vessels of the submucosal layer, due to hemodynamic conditions in the vessels of the microcirculatory bed, responded by increasing the diameter of both resistance and capacitance vessels, which is also reflected in the previous studies of vascular response to the impact of the complex of food additives on rat duodenal mucosa. Subsequently, the effect of food additives caused the development of inflammatory reaction with edema of the mucous membrane, which on week 8 was confirmed by a decrease in the diameter of the lumen of arterioles by 3.71 %, and a decrease in capillary diameter by 27.09 %, which led to activation of arteriovenous anastomoses for blood flow into the capacitance vessels, and as a consequence, to increase in the diameter of the venules of the mucous membrane. The restorative-compensatory reaction aimed at neutralizing the alternative factor and restoring the morphofunctional state of the vessels of the rat gastric fundus does not lead to complete restoration of hemodynamic conditions in the vessels of the mucous membrane and submucosal layer of the rat stomach, which leads to the phenomena of decompensation, a decrease in the diameter of the lumen of the exchange vessels, which in turn is expressed by hypoxia in the tissue of the gastric wall, and dilation of the capacitance vessels as a consequence of hemodynamic disorders.

Conclusion

The effect of the food additives complex on the vessels of the mucous membrane and submucosal layer of the rat gastric fundus at the early stages of the experiment is expressed by spasm of vessels of the microcirculatory bed of the mucous membrane due to direct impact of the components of food additives and enlarged diameters of submucosal vessels. Further development of the inflammatory response and the phenomenon of hypoxia led to the compensatory-restorative reaction, though complete recovery did not occur that, at the end of the experiment, was expressed by decompensation of the resistance vessels, spasm of the exchange vessels and enlargement of lumen of the capacitance vessels.

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