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G.A. Yeroshenko, A.S. Grygorenko, K.V. Shevchenko, O.D. Lysachenko, O.B. Riabushko, N.M. Pyvovar[1], O.V. Klepets
Poltava State Medical University, Poltava
[1]Poltava V.G. Korolenko National Pedagogical University, Poltava

INFLUENCE OF FOOD ADDITIVES COMPLEX ON THE MORPHOLOGY OF VILLI OF THE RATS' DUODENUM MUCOSA

e-mail: gala_umsa@ukr.net

The study presents the findings of the study of the effect of food additives, namely, monosodium glutamate, sodium nitrite and Ponceau-4R on the morphological state of the components of the villi of the rats' duodenal mucosa. The study was aimed at establishing the dynamics of changes in the metric parameters of the components of the villi of rats' duodenal mucosa in normal conditions and under the action of the complex of food additives. It has been found that consumption of the complex of food additives leads to changes in the metric values of the height and width of the villi, which at the early stages of the experiment leads to a decrease in their mean values due to the immediate direct action on the mucous membrane with subsequent development of inflammatory reaction and edema, which will lead to an increase in metric values and a decrease in the number of epithelial cells of the villus. Adaptive mechanisms do not fully restore the values of the morphometric study, which is manifested by a decrease in the height of the villi by 20.25 % compared with the control group with an increase in the number of enterocytes with brush border and a decrease in the number of goblet cells by 44.41 %.

Keywords: food additives, duodenum, villus, mucous membrane, rats.

Г.А. Єрошенко, А.С. Григоренко, К.В. Шевченко, О.Д. Лисаченко, О.Б. Рябушко, Н.М. Пивовар, О.В. Клепець

ВПЛИВ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК НА МОРФОЛОГІЮ ВОРСИН СЛИЗОВОЇ ОБОЛОНКИ 12-ПАЛОЇ КИШКИ ЩУРІВ

У роботі представлене дослідження впливу харчових добавок: глутамату натрію, нітриту натрію та Понсо-4R на морфологічний стан компонентів ворсин слизової оболонки 12-палої кишки щурів. Метою роботи було встановити динаміку змін метричних показників компонентів ворсин слизової оболонки дванадцятипалої кишки щурів в нормі та при дії комплексу харчових добавок. Встановлено, що вживання комплексу харчових добавок призводить до змін метричних показників довжини та ширини ворсин, що на ранніх стадіях експерименту призводить до зменшення їх середніх значень внаслідок безпосередньої прямої дії на слизову оболонку з наступним розвитком запальної реакції та набряком, які призведуть до збільшення значень метричних показників та зменшення кількості клітин епітелію ворсини. Адаптивно-пристосувальні механізми не повністю відновлюють показники морфометричного дослідження, що проявляється зменшенням довжини ворсин на 20,25 % порівняно з контрольною групою зі збільшенням кількості облямованих ентероцитів та зменшенням кількості келихоподібних клітин на 44,41 %.

Ключові слова: харчові добавки, 12-пала кишка, ворсина, слизова оболонка, щурі.

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A food additive is considered as any substance that is not normally a food product or its ingredient, though the production process it is added to a food product for technological purposes and becomes an integral part of the product [8].

Flavor enhancer, namely, monosodium glutamate (E-621), the sodium salt of glutamic acid, is the most common, the use of which reaches more than 200 thousand tons of glutamate annually. It is difficult to find manufactured pre-made food or fully made food that would not contain this additive [1, 9].

The findings of the study of the effect of monosodium glutamate on the gastric wall have found that in high doses, monosodium glutamate has a local pathogenic effect on gastric tissue, manifested in thinning of all layers of the gastric wall, desquamation of the mucous membrane and its disorganization by reducing the dimensions of the gastric glands [4].

Sodium nitrite (E-250) food additive is widely used as a color retainer in the manufacturing of meat products and a preservative to give products certain properties and maintain quality [10].

Sodium nitrite is reported to have harmful toxic effects on various organs of the body [6]. Chronic load of sodium nitrite, as shown in the work of Ukrainian scientists, induces the development of oxidative stress (elevated 2,3-bisphosphoglyceric acid), inflammation (elevated levels of interleukin-1-beta, which, in turn, causes a dramatic increase in iNOS activity), development of endothelial dysfunction (elevated Willebrand factor) [13].

In the food industry, the E-124 food additive is one of the most popular food colorants, giving the product an attractive appearance and is the initial stage of consumer interest [11]. Numerous azo dyes and their reduced products, as well as chemically bound aromatic amines, have been reported to affect human health, causing allergies and other human diseases [7]. The toxicity of azo dye ingredients increases with increasing benzene rings in their structure. The carcinogenicity of azo dyes directly depends on the structure of the molecule and the mechanism of degradation. The breakdown products of azo dyes are mainly aromatic amines with different structures, and they can have carcinogenic properties.

Apparently, the findings of the previous studies by Ukrainian and foreign scientists do not reveal the full picture of the effect of food additives, as they are most often used in combination. Therefore, the study of the effect of the complex of the most common food additives on the digestive system, which is the first to be in direct contact with an exogenous factor, is very relevant to date.

The purpose of the study was to establish the dynamics of changes in the metric parameters of the components of the villi of the rats' duodenal mucosa in normal conditions and under the action of the complex of food additives, namely, monosodium glutamate, sodium nitrite and Ponceau-4R.

Material and Methods. The study involved 84 outbred mature male rats. The control group of rats consumed drinking water and additionally received saline per os. The rats of the study group had access to water ad libitum and, supplementary, consumed 10 % sodium nitrite solution. Monosodium glutamate was administered at a dose of 20 mg/kg in 0.5 ml of distilled water; Ponceau-4R was given at a dose of 5 mg/kg in 0.5 ml of distilled water once daily per os. The doses of food additives half lowered the allowable normal rate in food products. The rats' adaptive behavior was evaluated using the "open field" test [15]. The resulting data were processed quantitatively by the methods of variation statistics using the Student's t-test and Excel software.

The rodents were sacrificed within 1, 4, 8 and 16 weeks under thiopentone anesthesia overdose. Upon euthanasia, the fragments of the duodenal wall were fixed in 10 % neutral formalin solution for three days. Subsequently, the pieces of the duodenal wall, fixed in formalin, were embedded into paraffin [12]. Slices of 5–10 μm thick were obtained using the sledge microtome and were mounted on the slides by stenciling technique. After hematoxylin and eosin staining, the slices were enclosed in polystyrene and studied in the light microscope. The digital microscope equipped with DCM 900 digital micro photo attachment and software, adapted to the studies, have been used for microimaging and morphometric study. Statistical processing of morphometric data was made using *Excel* software [2].

Results of the study and their discussion. A morphometric study of the duodenal mucosa revealed that the height and width of the villus of rats in the control group were 291.03±0.80 μm and 68.87±0.17 μm, respectively. The mean number of the columnar epitheliocytes with the brush border accounted for 18.00±0.08. Goblet exocrinocytes in the villus of rats of the control group were scattered among the columnar epitheliocytes with an average number of 11.01±0.08 (Table 1).

Table 1

Morphometric parameters of the mucosal villi

Parameters	Height	Wight	Quantitative composition of exocrinocytes in the villi		
	μm	μm	Absorptive exocrinocytes (in the FOV)	Goblet cells (in the FOV)	Intraepithelial lymphocytes (in the FOV)
Control	291.03±0.80	68.87±0.17	18.00±0.08	11.01±0.08	0.2±0.04
Week 1	229.99±1.09 *	85.85±0.59 *	17.67±0.09 *	3.00±0.08 *	0.5±0.05 *
Week 4	307.73±0.56 *,**	64.74±0.20 *,**	19.77±0.23 *,**	7.50±0.08 *,**	1±0.06 *,**
Week 8	180.50±0.61 *,**	42.09±0.20 *,**	17.00±0.08 *,**	4.00±0.08 *,**	2±0.09 *,**
Week 12	222.71±0.21 *,**	52.72±0.13 *,**	17.38±0.11 *,**	5.79±0.12 *,**	1.72±0.05 *,**
Week 16	232.09±0.48 *,**	70.43±0.15 *,**	18.51±0.09 *,**	6.12±0.16 *,**	2.64±0.05 *,**

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

In the lumen of the intestine, the digitiform projections of the mucous membrane, called the villi, were visualized, the basis of which was the lamina propria, formed mainly by the elements of loose fibrous connective tissue. The villi were covered mainly with columnar epitheliocytes with the brush border, between which goblet cells and endocrinocytes were scattered. The vascular complex of the microcirculation was located in the middle of the villus in the lamina propria. On the apical surface, the columnar epitheliocytes had a striped brush border formed by gazillion microvilli with glycocalyx on their

surface, represented by lipo- and glycoproteins. The structure of goblet exocrinocytes in the villus was represented by typical mucous cells that accumulated and excreted the products of secretion cyclically into the intestinal lumen. During the excretion phase, the goblet cell became narrow, its nucleus diminished, and the cytoplasm was without secretion granules (Fig. 1).

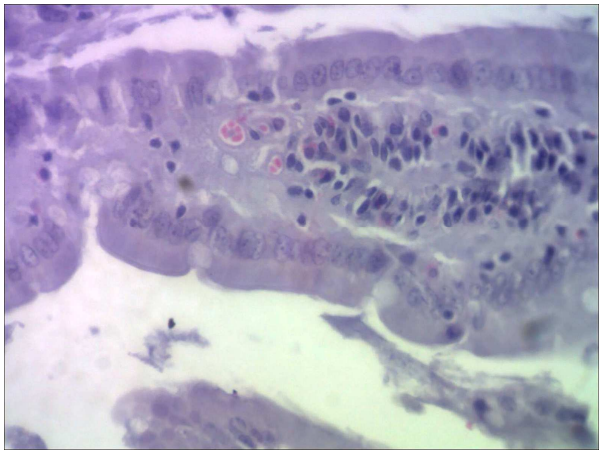

Fig. 1. The villus of the duodenal mucosa of control rats. H&E stain. Ocular lens: 10×magnification, objective lens: 40× magnification.

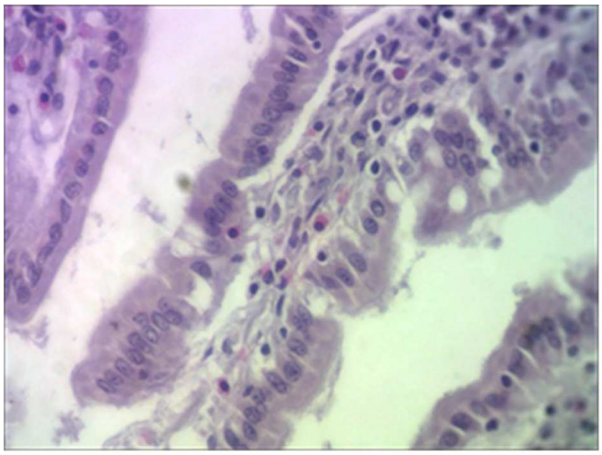

Fig. 2. The villus of the rats' duodenal mucosa on week 4 of consumption of a complex of food additives. H&E stain. Ocular lens: 10×magnification, objective lens: 40× magnification.

Within week 1 of consumption of a complex of food additives, the villi were shortened by 20.97 % ($p < 0.05$) with a mean value of 229.99±1.09 μm. The width of the villi increased by 24.66 % ($p < 0.05$), compared to the control group, with mean values of 85.85±0.59 μm. The average amount of columnar epitheliocytes accounted for 17.67±0.09 which was 1.83 % lower compared to the control group ($p < 0.05$). The number of goblet cells decreased significantly by 72.75 % ($p < 0.05$), with an average number of 3.00±0.08.

On week 4 of the experiment, the height of the villi was 307.73±0.56 μm, which was 33.80 % and 5.74 % ($p < 0, 05$) significantly greater compared with the parameters of week 1 and the value of the control group, respectively. The width decreased by 24.59 % compared to the results of the previous period of the experiment and accounted for 64.74±0.20 μm, which was 6.00 % lower than the control values ($p < 0.05$). The average number of columnar epitheliocytes increased by 11.89 % and 9.83 % compared with the previous period of the experiment and the value in the control group, respectively, accounting for 19.77±0.23 ($p < 0.05$). The average number of goblet exocrinocytes doubled in number on week 1 and accounted for 7.50±0.08, though was 31.88 % lower compared with the control group ($p < 0.05$). Intraepithelial lymphocytes were found with a mean value of 1/FOV.

The findings of the visual study of the morphological changes of the duodenum revealed that the columnar epitheliocytes with a striped brush border were elongated and prismatic, compacted and formed the main layer of cells from which the mucous membrane was formed. Microvilli with reduced density were located on the apical surface of these cells. Goblet exocrinocytes were located between columnar epitheliocytes with and without a striped brush border, their number in the field of view decreased, as a significant part of the cells were disintegrated (Fig. 2).

On week 8 of consumption of the complex of food additives, the mean values of the height of the villi decreased by 41.34 % and accounted for 180.50±0.61 μm, which was 37.98 % significantly lower than the value of the control group ($p < 0.05$). The width of the villi was 34.99 % and 38.88 % significantly lower compared with the values of the previous period of the experiment and the control group, respectively, and its mean values were 42.09±0.20 μm ($p < 0.05$). The average number of columnar enterocytes decreased by 14.01 % and 5.56 % compared to the values on week 4 (17.00±0.08) and the values of the control group, respectively ($p < 0.05$). The average number of goblet cells was 41.33 % lower than the values of the previous period of the experiment and accounted for 4.00±0.08, which was 63.67 % significantly lower than the values of the control group ($p < 0.05$). Intraepithelial lymphocytes were determined with the mean value of 2/FOV.

Enterocytes with the brush border were elongated and prismatic, densely organized; some of them had an irregular shape, the striped brush border was disintegrated. The sites with impaired contact between cells were detected. The number of goblet cells visually decreased, they were located between the bases of enterocytes and were at the stage of accumulation. Lymphocytes with a basophilic nucleus and a narrow rim of cytoplasm and plasma cells were visualized in the depth of the epithelial layer of the villus (Fig. 3).

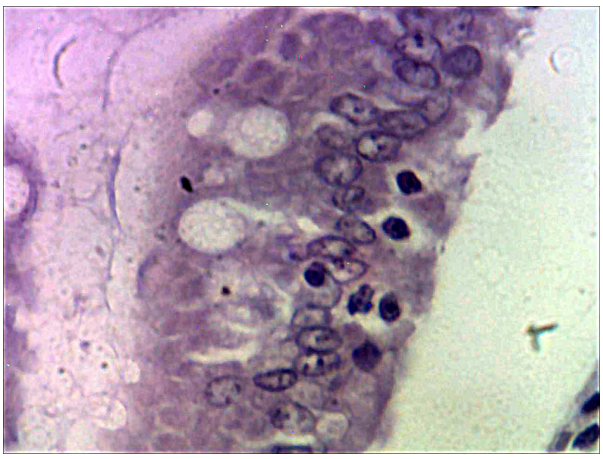

Fig. 3. Intraepithelial lymphocyte and plasma cells of the villus of the rats' duodenal mucosa on week 8 of consumption of the complex of food additives. H&E stain. Ocular lens: 10×magnification, objective lens: 100×magnification.

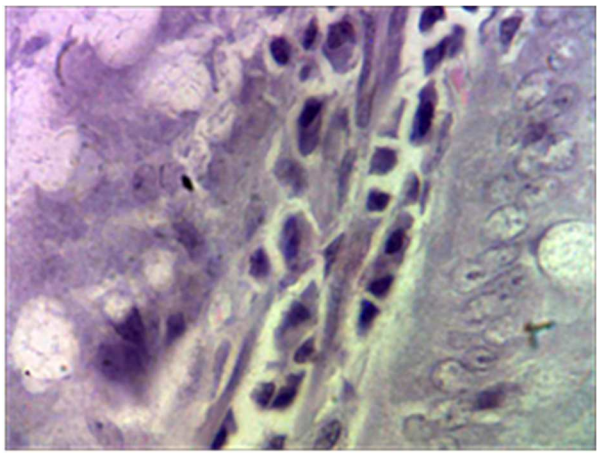

Fig. 4. Leukocyte infiltration of the villus of the rats' duodenal mucosa on week 16 of the experiment. H&E stain. Ocular lens: 10×magnification, objective lens: 100×magnification.

The consumption of complex of food additives of monosodium glutamate, sodium nitrite and Ponceau-4R on week 12 led to an increase in the mean values of the height of the villi of the rats' duodenal mucosa by 23.39 %, compared to the previous period of the experiment, and accounted for 222.71±0.21 µm; however, these values were by 23.48 % significantly lower than the value of the control group ($p < 0,05$). The width of the villi increased by 25.26 %, compared with the values of week 8, accounting for 52.72±0.13 µm, and was also by 23.45 % significantly lower than the mean values of the control group ($p < 0.05$). The number of enterocytes with the brush border increased by 2.24 % compared to the previous study period, but was by 3.44 % lower than the value in the control group with mean values of 17.38±0.11 ($p < 0.05$). The average number of goblet cells on week 12 accounted for 5.79±0.12, which was by 44.75 % significantly greater than the value of the previous period of the experiment, though was by 47.41 % significantly lower than their value in the control group of animals ($p < 0.05$). The number of intraepithelial lymphocytes was 1-2/FOV.

On week 16 of the experiment, the mean values of the height of the villi of the duodenal mucosa were 232.09±0.48 µm, which was by 4.21 % significantly greater compared with the results of the previous period of the experiment, though was by 20.25 % significantly lower than the values of the controls ($p < 0.05$). The width of the villi significantly increased by 33.59 % and accounted for 70.43±0.15 µm, though was by 2.27 % significantly lower than the values of the control group ($p < 0.05$). The average number of enterocytes with the brush border increased by 6.50 % and 2.83 % compared with the values on week 12 of the experiment and control group of rats, respectively, accounting for 18.51±0.09 ($p < 0.05$) on week 16. The number of goblet cells accounted for 6.12±0.16 and was by 5.78 % significantly greater the results of the previous period of the experiment that was by 44.41 % significantly lower than the values of the control group of animals ($p < 0.05$). The number of intraepithelial lymphocytes was 3/FOV.

The findings of the histological study revealed a large number of plasma cells in the lamina propria, located in groups and forming chains of 4–6 cells. Intraepithelial lymphocytes were found in the epithelium (Fig. 4).

Thus, the effect of the combination of food additives of monosodium glutamate, sodium nitrite and Ponceau-4R develops a complex, local vascular-mesenchymal reaction, which aims to neutralize the action of the alternative agent and restore the morphofunctional state of the duodenal wall, including the villi. At the early stages of the experiment, a decrease in the mean values of morphometric parameters of the height of the villi occurs, which leads to an increase in their width, with a decrease in the number of enterocytes with the brush border and goblet cells, and was primarily due to the immediate direct action of chemicals on the mucosa. This phenomenon is confirmed by the experiments of Ukrainian researchers in studying the effect of exogenous factors on mucous membranes with changes in morphometric parameters and morphological structure [5]. Subsequently, an increase in the height of the villi of the mucous membrane occurs with a decrease in the mean values of the width, due to the edema development in response of the action of the alternative factor with the events of inflammation, as evidenced by increased secretion by the degranulated goblet cells, some of them were disintegrated, and disrupted striped brush border of absorbing enterocytes. The above impairments are caused by microcirculation disorders that occur under the influence of toxic exogenous factors [14]. On week 8, intensification of the regenerative processes, caused by the development of adaptive mechanisms, was noted, which initially led to a decrease in the mean metric values

and their gradual recovery during the experiment.The action of the complex of food additives causes tension of the local protective barrier, as evidenced by an increase in the number of intraepithelial lymphocytes from 1 to 3/FOV during the experiment and an increase in the number of plasma cells in the lamina propria, which formed groups and chains [3]. At the end of the experiment, the metric parameters were not restored, the height of the villi was 20.25 % significantly lower than the control values, which accordingly led to a slight increase in the mean values of the width of the villi by 2.27 %. This phenomenon led to an increase in the average number of enterocytes with brush border due to a decrease in the height of the villi to ensure the processes of parietal digestion and absorption, which increased by 2.83 % compared to the control group, the number of goblet cells remained significantly lower during the experiment, since they are the final stage of differentiation of enterocyte differon, and on week 16 their number was by 44.41 % lower than the control values.

Conclusion

Thus, the effect of the complex of the food additives of monosodium glutamate, sodium nitrite and Ponceau-4R on the duodenal mucosa leads to changes in the metric parameters of the height and width of the villi, which at the early stages of the experiment leads to a decrease in their mean values due to immediate direct action on the mucosa with the subsequent development of inflammatory reaction and edema, which will lead to an increase in metric values and a decrease in the number of epithelial cells of the villus. Adaptive mechanisms do not fully restore the values of the morphometric study, which is manifested by a decrease in the height of the villi by 20.25 % compared with the control group with an increase in the number of enterocytes with brush border and a decrease in the number of goblet cells by 44.41 %.

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