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REACTION OF THE CAPACITIVE LINK OF THE HEMOMICROCIRCULATORY BED OF THE ILEUM UNDER OXIDATIVE STRESS CAUSED BY THE INTRODUCTION OF A COMPLEX OF CHEMICAL FOOD ADDITIVES

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Food chemical additives are common exogenous factors that can cause changes in the morphology and biochemical parameters of various organs, including the ileum. Therefore, the purpose of our study was to determine changes in the capacitive link of the hemomicrocirculatory bed of the rat ileum and fluctuations in malondialdehyde levels after complex administration of monosodium glutamate, sodium nitrite, and Ponceau 4R. Histological and morphometric examination of the venules of the mucosa and submucosa, as well as determination of the concentration of malondialdehyde in the ileum homogenate of rats of the control group (receiving saline) and experimental groups (administered the test mixture for 1, 4, 8, 12, 16, 20 weeks) were performed. When assessing changes in morphometric parameters, the reaction of the capacitive link of the hemomicrocirculatory bed was revealed, in particular, vasoconstriction of venules from 1 to 8 weeks, partial recovery to control values at 12 weeks and significant vasodilation after 16 weeks, which persisted until the end of the experiment. Regarding biochemical changes, it was found that introducing a complex of chemical food additives leads to signs of oxidative stress, which was confirmed by an increase in the concentration of malondialdehyde. Thus, it was determined that oxidative stress is one of the pathogenetic mechanisms of morphometric changes in the vessels of the capacitive link.

Key words: small intestine, hemomicrocirculatory bed, venules, food additives, rats, mucosa, submucosa, oxidative stress, malondialdehyde, morphometry.

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РЕАКЦІЯ ЄМНІСНОЇ ЛАНКИ ГЕМОМІКРОЦИРКУЛЯТОРНОГО РУСЛА КЛУБОВОЇ КИШКИ ПРИ ОКСИДАТИВНОМУ СТРЕСІ, ВИКЛИКАНОМУ ВВЕДЕННЯМ КОМПЛЕКСУ ХІМІЧНИХ ХАРЧОВИХ ДОБАВОК

Харчові хімічні добавки є поширеними екзогенними чинниками, що можуть викликати зміни морфології та біохімічних показників різних органів, зокрема клубової кишки. Тому метою нашого дослідження було визначення змін показників ємнісної ланки гемомікроциркуляторного русла клубової кишки щурів та коливання рівня малонового діальдегіду на тлі комплексного введення глутамату натрію, нітриту натрію та понсо 4R. Було проведено гістологічне та морфометричне дослідження венул слизової та підслизової оболонок, а також визначення концентрації малонового діальдегіду у гомогенаті клубової кишки щурів контрольної групи (отримувала фізіологічний розчин) та експериментальних груп (яким вводили досліджувану суміш протягом 1, 4, 8, 12, 16, 20 тижнів). При оцінці змін морфометричних показників було виявлено реакцію ємнісної ланки гемомікроциркуляторного русла, зокрема вазоконстрикція венул з 1 по 8 тиждень, часткове відновлення до контрольних показників на 12 тижні і значна вазодилятація після 16 тижня, що зберігалася до кінця експерименту. Щодо біохімічних змін, то було встановлено, що введення комплексу хімічних харчових добавок призводить до виникнення ознак оксидативного стресу, що підтверджувалося підвищенням концентрації малонового діальдегіду. Таким чином, було визначено, що оксидативний стрес є одним з патогенетичних механізмів виникнення морфометричних змін судин ємнісної ланки.

Ключові слова: тонка кишка, гемомікроциркуляторне русло, венули, харчові добавки, щури, слизова оболонка, підслизова оболонка, оксидативний стрес, малоновий діальдегід, морфометрія.

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Food additives are widely used in the food industry for several main reasons. They can improve the flavouring properties of products by adding a specific taste or aroma and facilitating the production of various flavour combinations. Food additives are also used to extend the shelf life of products, preventing spoilage and keeping them fresh on store shelves. In addition, additives can preserve or even improve the colour of products, making them more attractive to consumers. In order to achieve the best effect, the modern food industry is characterized by the complex use of several food additives at the same time. These substances can be of natural or chemical origin. According to the results of several scientific studies, the latter can cause morphological changes in internal organs, particularly the digestive system [4, 6, 8, 10]. The consequence of these changes is a violation of their functional properties. The problem of determining the pathogenetic mechanisms of their occurrence is a highly relevant problem of modern science, especially when it comes to such an essential part of the digestive tract as the ileum, which not only performs several important digestive processes but also has a complex morphological structure.

Changes in biochemical parameters, particularly the development of oxidative stress, often cause the remodelling of internal organs' structural elements. An important marker of which in the organ homogenate is the level of malondialdehyde (MDA), a product of the breakdown of polyunsaturated fatty acids under the influence of reactive oxygen species (ROS). MDA is a part of the lipid oxidation process and is highly reactive, which allows it to interact with other molecules in the body, including proteins and DNA. Fluctuations in the body's MDA level can occur in inflammatory processes, chronic diseases, stress, increased physical activity, and the toxic effects of exogenous factors, including chemical food additives [7, 11].

Therefore, establishing morphological changes in the ileum and fluctuations in MDA levels accompanying them against the background of the complex use of chemical food additives is an important task for modern science, particularly in the context of finding ways to correct their negative impact.

The purpose of the study was to determine the changes in morphometric parameters of the capacitive link of the hemomicrocirculatory bed of the rats' ileum and fluctuations in the level of malondialdehyde against the background of complex administration of monosodium glutamate, sodium nitrite and ponceau 4R.

Materials and methods. The study was conducted on 70 sexually mature white rats weighing 180 to 252 g, which were kept in standard conditions of the vivarium of Poltava State Medical University. The rats were divided into control and six experimental groups of 10 animals each. Animals of the control group received saline, and animals of the experimental groups received a mixture of chemical food additives (monosodium glutamate, sodium nitrite, ponceau 4R) in single doses: 20 mg/kg of monosodium glutamate, 5 mg/kg of Ponceau 4R and 0.6 mg/kg of sodium nitrite in 0.5 ml of distilled water orally, for 1, 4, 8, 12, 16 and 20 weeks. These doses were half the maximum permissible doses for food. At all other times, rats had free access to water and standard vivarium food. The experimental animals were withdrawn from the experiment by an overdose of sodium thiopental, and then experimental material was collected for histological and biochemical studies. Biopsies of the ileum were sealed in paraffin and epoxy resin, followed by the preparation of histological sections and their staining with hematoxylin and eosin, according to Hart, Van Gieson, methylene blue according to generally accepted methods [2]. The histological specimens were examined using a Levenhuk D740T microscope with a digital microphotomount and programs adapted for these studies. The following morphometric parameters of the venules of the mucosa and submucosa of the ileum were determined: mean total diameter, mean lumen diameter, mean wall thickness.

The concentration of malondialdehyde in the ileum homogenate was determined by a method based on the ability of free MDA to specifically interact with 1-methyl-2-phenyl-indole in a mixture of methanol and acetonitrile. This interaction formed an orange chromogen (carbocyanine dye) with a maximum absorption at a wavelength of 586 nm.

The statistical analysis of the results was performed using a personal computer and the InStat software package, which is used for statistical processing of data from biomedical and epidemiological studies. The difference was considered statistically significant if $p<0.05$.

The study was conducted in accordance with the "Rules for the Use of Laboratory Experimental Animals" (2006, Appendix 4) and the Helsinki Declaration for the Humane Treatment of Animals, the Law of Ukraine "On the Protection of Animals from Cruelty" (No. 3447-IV of 21.02.2006).) in compliance with the requirements of the Bioethics Commission of Poltava State Medical University (Protocol No. 208 of 22.09.2022), following the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

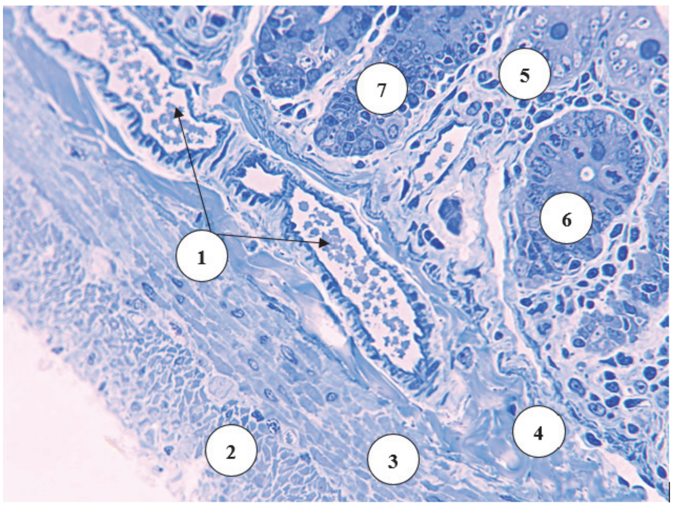

Fig. 1. Structural components of the rats' ileum of the control group. Semi-thin section. Methylene blue staining. Magnification: okh. 10; obh. 40. 1. Venule; 2. Serosa; 3. Muscular layer; 4.Submucosa; 5. Mucosa; 6. Crypt; 7. Intestinal villi.

Results of the study and their discussion. The capacitive link of the hemomicrocirculatory bed of the rats' ileum of the control and experimental groups is represented by venules. These vessels have a thin wall formed by a layer of endothelial cells on the basement membrane and adventitial fibroblasts (Fig. 1).

When analyzing the mean value of the total diameter of the submucosal venules, it was found that this indicator after 1 week of administration of the complex of chemical food additives statistically significantly decreased by 9.75 % compared to the control, after 4 weeks – by 17.92 % compared to the control and by 9, 06 % compared to the previous experimental period, after 8 weeks a similar dynamics was observed, characterized by a decrease in the studied indicator by 38.99 % compared to the corresponding indicator of the control group and by 25.67 % compared to the previous observation period, at $p<0.05$. After the 12th week of the experiment, the mean total diameter of the vessels, although 18.87 % less than the control group, increased by 32.99 % compared to the previous period. The highest morphometric values of the studied indicator were established after 16 weeks of administration of the complex of chemical food additives. They were characterized by an increase of 72.64 % compared to the control group and 112.79 % compared to the previous study period, at $p<0.05$. After 20 weeks, the indicator was 4.4 % higher than the control group but 39.53 % lower than in the previous term of the experiment (Fig. 2A).

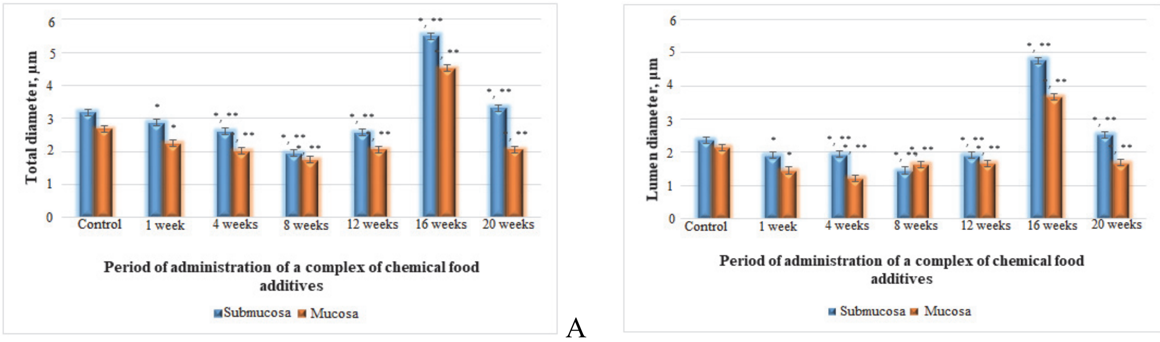

Fig. 2. Dynamics of changes in the mean values of the total diameter of the venules (A) and the mean lumen diameter of the venules (B) of the submucosa and mucosa of the rats' ileum at different periods of administration of a complex of chemical food additives. Note: * – statistically significant at $p<0.05$ compared to the control group, ** – statistically significant at $p<0.05$ compared to the previous observation period.

As for the morphometric analysis of the mean lumen diameter of the venules of the ileum's submucosa, the following changes were found. After 1 week of administration of the complex of chemicals, this indicator decreased by 19.4 % compared to the control. After 4 weeks of the study, a statistically significant decrease of 18.14 % was found compared to the control, with a slight increase of 1.57 % compared to the previous study period (at $p<0.05$). After 8 weeks, the mean diameter of the venous lumen decreased both in the control group and in the previous experimental period and was 38.82 % and 25.26 % smaller, respectively. After 12 weeks, an increase of 31.72 % was observed compared to the previous period, although it was 19.4 % less than in the control group. At the next stage of the study (after 16 weeks of administration of monosodium glutamate, sodium nitrite and Ponceau 4R), a simultaneous increase in the study indicator was found both in comparison with the control and the previous period, in particular by 100.84 % and 149.21 %, respectively. After week 20 of the experiment, a slight increase in the indicator was found compared to the control group (by 6.75 %), with a simultaneous decrease compared to the previous experimental period (46.84 %), at $p<0.05$ (Fig. 2B).

The analysis of the mean wall thickness of the venules of the submucosa of the rats' ileum showed that after 1 week of administration of a complex of chemical food additives to experimental animals, the indicator decreased by 7.89 % compared to the control, after 4 weeks – by 15.79 % compared to the control group and by 8.57 % compared to the previous period, at $p<0.05$. After 8 weeks, the indicator increased, compared to the control and the previous period, by 2.63 % and 21.86 %, respectively. After week 12, a similar trend was observed – an increase in the indicator by 15.79 % and 12.82 %, respectively. A further increase in vascular wall thickness was detected after week 16, mainly by 31.85 % compared to the control and 13.64 % compared to the previous period, at $p<0.05$. After week 20, the indicator was 21.05 % higher than in the control group but 8 % lower than in the previous experimental group (Fig. 3).

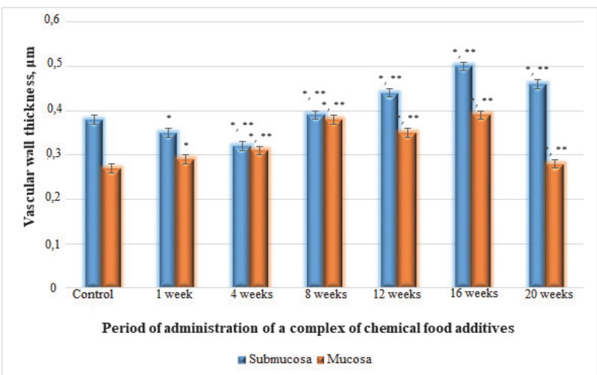

Fig. 3. Dynamics of changes in the mean wall thickness of the venules of the submucosa and mucosa of the rat's ileum at different periods of administration of a complex of chemical food additives. Note: * – statistically significant at $p<0.05$ compared to the control group, ** – statistically significant at $p<0.05$ compared to the previous observation period.

Subsequently, a morphometric analysis of the capacitive link of the ileal mucosa was performed.

Thus, the mean total diameter of the venules of this layer after 1 week of the study was 16.72 % smaller than in the control group. After 4 weeks, the indicator decreased by 25.28 % compared to the control and 10.27 % compared to the previous period ($p<0.05$). A further decrease was found after week 8, particularly by 34.94 % and 19.94 %, respectively. After the 12th week of the experiment, the studied indicator was 23.42 % lower than the control but 17.71 % higher than in the previous term. A sharp increase was found after week 16, in particular, by 67.66 % compared to the control and 118.93 % compared to the previous study stage, at $p<0.05$. After week 20, the indicator was 23.42 % lower than in the control group and 54.32 % lower than in the previous experimental group.

Analyzing the mean lumen diameter of the venules of the ileal mucosa, it was found that after 1 week of the study, it decreased by 32.56 % compared to the control. After 4 weeks, a further decrease was observed, particularly by 43.25 % compared to the control and 15.86 % compared to the previous observation period. After 8 weeks, it was found that the indicator was 24.19 % lower than in the control group but 33.61 % higher than in the previous period, at $p<0.05$. After week 12, the indicator was 22.33 % lower than the control group but slightly higher than in the previous term (by 2.45 %). A sharp increase in the studied indicator compared with the control group and the previous stage of the study was found after week 16, by 70.7 % and 119.76 %, respectively ($p<0.05$). After week 20, the mean lumen diameter of the venules of the ileal mucosa was 21.39 % lower than in the control group and 53.95 % lower than in the previous experimental group.

The following changes were found in the analysis of the mean thickness of the venous wall of the ileal mucosa. After 1 week of administration of the experimental mixture, the indicator increased by 7.4 % compared to the control group. After 4 weeks, the indicator increased by 14.81 % compared to the control and by 6.89 % compared to the previous period ($p<0.05$). After week 8, there was a further increase in the indicator, particularly by 40.74 % and 22.58 %. After week 12, although the study indicator was 29.63 % higher, it was 7.89 % lower than at the previous study stage. After week 16, changes in the indicator were observed in the direction of growth, particularly by 44.44 % compared to the control and by 11.43 % compared to the previous period, at $p<0.05$. After week 20, the mean thickness of the vascular wall of the mucosal venule was 3.7 % greater than in the control group but 28.2 % less than at the previous stage.

The next stage of the study was to determine changes in the concentration of MDA in the ileum homogenate at different periods of administration of monosodium glutamate, sodium nitrite and ponceau 4R in combination. Thus, it was found that after 1 week, the studied indicator statistically significantly increased by 52.4 % compared to the control group. After week 4 of the study, the concentration of MDA increased by 27.86 % compared to the control group but was 16.1 % lower than at the previous stage. After week 8, the indicator was 25.52 % higher than the control group but 1.83 % lower than at the earlier study stage, at $p<0.05$. After week 12, an increase in MDA concentration was observed in the control and the previous experimental group, in particular by 40.57 % and 11.98 %, respectively. After week 16, the indicator increased again – 81.75 % compared to the control group and 29.3 % compared to the previous observation period ($p<0.05$). The highest concentration of malondialdehyde was found after week 20. It was 105.92 % higher than in the control group and 13.3 % higher than in the previous period (Fig. 4).

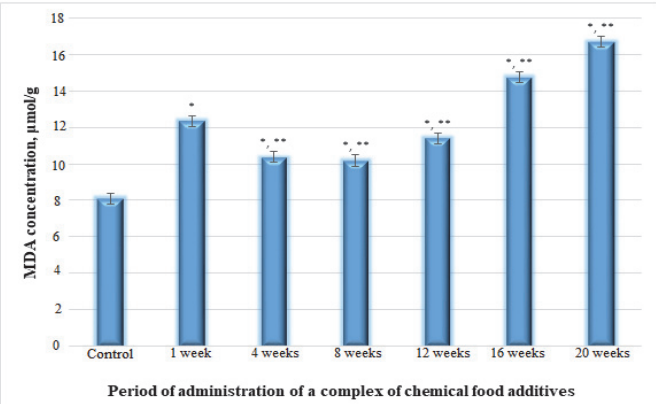

Fig. 4. Dynamics of changes in the mean concentration of malondialdehyde in the rats' ileum at different periods of administration of a complex of chemical food additives. Note: * – statistically significant at $p<0.05$ compared to the control group, ** – statistically significant at $p<0.05$ compared to the previous observation period.

We have found that the complex administration of monosodium glutamate, sodium nitrite and ponceau 4R causes significant metric changes in the vessels of the capacitive link of the hemomicrocirculatory bed of the submucosa and mucosa of the ileum in rats in the dynamics of the experiment. Thus, analyzing changes in the mean total diameter of venules and the mean lumen diameter of both submucosa and mucosa, it was found that at the initial stages of the experiment (up to 8 weeks), vasoconstriction phenomena were observed, which are most likely due to the primary reaction of blood vessels to the damaging agent and are the initial manifestation of compensatory and recovery processes. After 12 weeks, signs of vasodilation appear, which are most pronounced after 16 weeks and slightly decrease after 20 weeks, but without returning to the control values, which, in our opinion, is a manifestation of the cumulative damaging effect of an exogenous factor, and is also caused by metric changes in the intestinal wall [5]. As for the changes in the mean thickness of the vascular wall, they are dynamic, with fluctuations both in the direction of a slight decrease due to the external pressure of the thickened and edematous intestinal wall and the thickening of the venous wall as a result of the direct damaging effect of the exogenous factor on its endothelial layer.

Evaluating the changes in MDA concentration, it was found that there was a sharp increase in the first week, which was the primary reaction to chemicals. In our opinion, the decrease in MDA levels after 4 and 8 weeks is associated with the activation of antioxidant mechanisms aimed at restoring balance and reducing oxidation. However, continued exposure to the pathogenic factor in the form of a complex of chemical food additives causes a weakening of antioxidant systems and, as a result, further damage and new oxidation cycles. Thus, there is a disruption of oxidation and reduction processes. It results in oxidative stress. In our opinion, this is one of the main pathogenetic mechanisms underlying the metric changes in the capacitive link of the hemomicrocirculatory bed of the rat’s ileum that we have identified.

Other studies have shown that vascular structures respond to other exogenous factors [1, 3]. Regarding the complex effect of monosodium glutamate, sodium nitrite, and ponceau 4R, it was found that their administration causes changes in the hemomicrocirculatory bed in various parts of the digestive tract [10, 14] and other systems [13].

Conclusion

The complex administration of monosodium glutamate, sodium nitrite and ponceau 4R for a long period leads to the development of signs of oxidative stress, which is the pathogenetic basis of the disorder of microcirculation of the capacitive link of the hemomicrocirculatory bed in the submucosa and mucosa of the ileum, characterized by vasoconstriction of venules from week 1 to 8, partial recovery to control values at week 12, and significant vasodilation after week 16, which persists until the end of the experiment. These changes are a compensatory and restorative response to oxidative stress caused by introducing chemical food additives.

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