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MORPHOLOGICAL AND FUNCTIONAL CHANGES IN THE EXCHANGE VESSELS OF THE RAT ILEAL MUCOSA UNDER NITRITE ACCUMULATION INDUCED BY COMBINED ADMINISTRATION OF MONOSODIUM GLUTAMATE, SODIUM NITRITE, AND PONCEAU 4R

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Chemical food additives, especially when administered in combination, can induce morphofunctional changes in various organs, including the ileum. Therefore, the aim of this study was to evaluate alterations in the morphometric parameters of the exchange segment of the haemomicrocirculatory bed in the ileal mucosa of rats, as well as fluctuations in nitrite levels, following the combined administration of monosodium glutamate, sodium nitrite, and Ponceau 4R. To achieve this, histological and morphometric methods were employed, alongside the determination of nitrite concentrations in biopsies of the target organ from control animals (administered saline) and experimental groups, which received the mixture of food additives for 1, 4, 8, 12, 16, and 20 weeks. The obtained results were subjected to statistical analysis. It was established that alterations in the mean values of the total diameter, lumen diameter, and wall thickness of capillaries occurred from the early stages of the study. These parameters exhibited dynamic fluctuations, with the most pronounced changes observed in the vascular wall, which demonstrated degenerative alterations. A statistically significant increase in nitrite concentration was noted in the biochemical assessments, persisting throughout the entire duration of the experiment. Thus, it was concluded that the combined administration of chemical food additives induces marked structural and biochemical changes in the ileum.

Key words: small intestine, haemomicrocirculatory bed, capillaries, food additives, rats, mucosa, nitrites, morphometry.

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МОРФОФУНКЦІОНАЛЬНІ ЗМІНИ СУДИН ОБМІННОЇ ЛАНКИ СЛИЗОВОЇ ОБОЛОНКИ КЛУБОВОЇ КИШКИ ЩУРІВ НА ТЛІ НАКОПИЧЕННЯ НІТРИТІВ, ВИКЛИКАНОГО КОМПЛЕКСНИМ ВВЕДЕННЯМ ГЛУТАМАТУ НАТРІЮ, НІТРИТУ НАТРІЮ ТА ПОНСО 4R

Хімічні харчові добавки, особливо при комплексному застосуванні, можуть викликати морфофункціональні зміни різних органів, зокрема клубової кишки. Тому метою дослідження було оцінити зміни морфометричних показників обмінної ланки гемомікроциркуляторного русла слизової оболонки клубової кишки щурів та коливання рівня нітритів при введенні глутамату натрію, нітриту натрію та понсо 4R у комплексі. Для досягнення мети було використано гістологічний та морфометричний методи, а також визначення концентрації нітритів у біоптатах досліджуваного органу тварин контрольної (вводили фізіологічний розчин) та експериментальних груп, які отримували дослідну суміш хімічних харчових речовин протягом 1, 4, 8, 12, 16 та тижнів. Отримані результати опрацьовували статистично. Було визначено, що зміни середніх показників зовнішнього діаметру, діаметру просвіту та товщини стінки капілярів виникали вже на ранніх термінах дослідження. Коливання показників мали динамічний характер, а найбільш вираженими бути зміни судинної стінки, які мали дегенеративний характер. При оцінці біохімічних показників, то було встановлено статистично достовірне підвищення концентрації нітритів, що прогресувало до кінця експерименту. Таким чином, було визначено, що введення комплексу хімічних харчових добавок викликає суттєві зміни клубової кишки на структурному та біохімічному рівнях.

Ключові слова: тонка кишка, гемомікроциркуляторне русло, капіляри, харчові добавки, щури, слизова оболонка, нітрити, морфометрія.

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The modern food industry extensively employs various additives to enhance flavour, preserve colour, extend shelf life, and improve the overall appeal of products. These substances may be of either natural or synthetic origin. Among chemical additives, the most commonly used are monosodium glutamate (E621), sodium nitrite (E250), and the dye Ponceau 4R (E124). Although the use of these compounds is regulated and limited to permissible doses, numerous experimental studies suggest their potential adverse effects not only on the digestive tract but also on other body systems [7, 13]. The concurrent use of multiple chemical additives is a widespread practice, which may amplify toxic effects and increase the risk of harmful outcomes. The literature reports the detrimental impact of the combined administration of monosodium glutamate, sodium nitrite, and Ponceau 4R on the lungs [14], duodenum [15], and other organs.

The ileum plays a key role in the processes of digestion and nutrient absorption. Its mucosa possesses a well-developed haemomicrocirculatory system, which ensures epithelial trophism and facilitates local immune responses. Disruption of the functional state of the exchange vessels may result in

ischaemia, hypoxia, inflammation, and dystrophic-necrotic changes. Owing to the complex structural organisation of the ileal mucosa, it is often a target organ upon exposure to exogenous factors, particularly chemical food additives [5].

A common cause of such changes may be biochemical imbalances, particularly elevated nitrite levels in tissues. Increased concentrations of nitrites may be accompanied by endothelial dysfunction, impaired vascular wall permeability, local oedema, hypoxia, and activation of inflammatory processes [1, 3]. These conditions contribute to the remodelling of the haemomicrocirculatory bed and the development of morphological alterations in internal organs, particularly in the ileal mucosa, especially under prolonged dietary exposure to chemical additives.

Therefore, investigating the combined effects of chemical food additives on the haemomicrocirculatory bed, especially its exchange segment, is essential for understanding the pathogenetic mechanisms of their action and for the rational assessment of potential health risks associated with their chronic consumption.

The purpose of the study was to assess morphological changes in the exchange segment of the haemomicrocirculatory bed of the ileal mucosa in rats, as well as fluctuations in nitrite levels, following the combined administration of monosodium glutamate, sodium nitrite, and Ponceau 4R.

Materials and methods. The study was conducted on 70 sexually mature white mongrel rats weighing 180–252 g, housed under standard conditions in the vivarium of Poltava State Medical University. The animals were divided into a control group and six experimental groups, each comprising 10 individuals. The control group received saline, while the experimental groups were administered a mixture of food additives comprising monosodium glutamate (20 mg/kg), Ponceau 4R dye (5 mg/kg), and sodium nitrite (0.6 mg/kg), dissolved in 0.5 ml of distilled water. The mixture was administered orally once, with exposure durations of 1, 4, 8, 12, 16, and 20 weeks. The selected doses corresponded to 50 % of the maximum permissible levels established for food products. Throughout the study period, the animals had ad libitum access to water and a standard vivarium diet.

Biomaterial sampling was performed following euthanasia of the rats via thiopental sodium overdose. Fragments of the ileum were collected for subsequent morphological and biochemical analyses. The obtained biopsies were fixed, dehydrated, and embedded in paraffin and epoxy resin using standard protocols, after which histological sections were prepared. The sections were stained with haematoxylin and eosin, as well as by Hart's, Van Gieson's, and methylene blue methods, in accordance with established histological procedures [2]. Morphological changes were examined using a Levenhuk D740T microscope equipped with a digital microphotography system and appropriate software. Morphometric parameters, including mean total diameter, mean lumen diameter, and mean wall thickness of the capillaries in the ileal mucosa, were assessed.

The concentration of nitrites was determined by detecting diazo compounds formed in the reaction with sulfanilic acid, followed by a reaction with α-naphthylamine (Griess-Ilosvay reagent). This reaction produces red-coloured derivatives (azo dyes), the intensity of which is proportional to the nitrite concentration [1].

The results were processed using a personal computer and the InStat software package, designed for statistical analysis in medical, biological, and epidemiological research. Statistical significance was set at $p<0.05$.

All stages of the study were conducted in accordance with the Rules for the Use of Laboratory Experimental Animals (2006, Appendix 4), the Helsinki Declaration on the Humane Treatment of Animals, and the Law of Ukraine On the Protection of Animals from Cruel Treatment (No. 3447-IV, 21 February 2006). The study was approved by the Bioethics Committee of Poltava State Medical University (Protocol No. 208, 22 September 2022) and complies with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

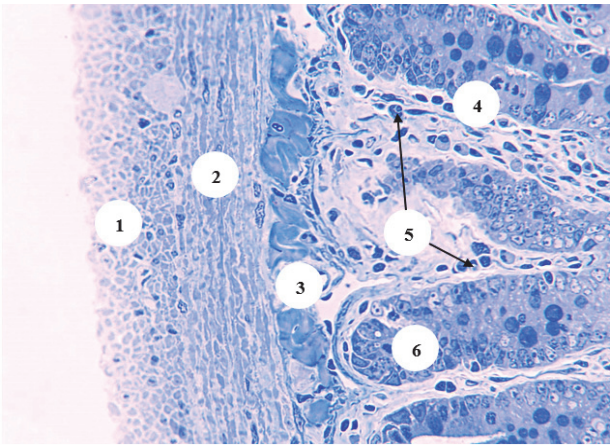
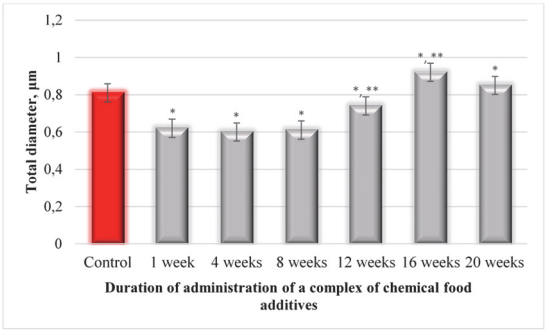


Fig. 1. Structural organisation of the ileum in control group rats. Semi-thin section. Methylene blue staining. Magnification: okh. 10; obh. 40. 1. Serosa; 2. Muscular layer; 3. Submucosa; 4. Mucosa; 5. Capillary. 6. Crypt.

Results of the study and their discussion. The vessels of the exchange segment in the haemomicrocirculatory bed of the rat ileal mucosa in both control and experimental groups consist of capillaries. These vessels have very thin walls composed of endothelium, basement membrane, and pericytes (Fig. 1).

Analysing the mean values of total capillary diameter, it was determined that after 1 week of administering monosodium glutamate, sodium nitrite, and Ponceau 4R, this parameter decreased by 23.46 % compared to the control group. After 4 weeks, a further decrease of 3.23 % compared to the previous time point and of 25.93 % compared to the control was observed ($p<0.05$). After 8 weeks, the mean value of the morphometric parameter increased by 1.61 % relative to the previous measurement but remained 24.69 % lower than the control. After 12 weeks, the parameter increased by 21.31 % compared to week 8 but was still 8.64 % lower than the control ($p<0.05$). After 16 weeks, the mean total capillary diameter increased markedly compared to both the previous time point and the control group, by 24.32 % and 13.58 %, respectively. At the final stage of administration (20 weeks), the parameter decreased by 7.61 % compared to week 16 but remained 4.94 % higher than the control (Fig. 2A).

When analysing the mean values of the diameter of capillary lumens in the ileal mucosa following the administration of a complex of chemical food additives, it was found that after 1 week of the study, the indicator decreased by 5.17 % compared to the control. After 4 weeks, there was a sharp decrease in the indicator relative to both the previous stage and the control group, by 21.82 % and 25.86 %, respectively ($p<0.05$). After 8 weeks, the morphometric parameter increased by 30.23% compared to the previous period but remained 3.45 % lower than the control. At 12 weeks, the mean diameter of the capillary lumen increased by 21.43 % compared to the previous period and by 17.24 % compared to the control group, both at $p<0.05$. At the next stage of the experiment (after 16 weeks), this parameter decreased by 2.94 % compared to week 12 but was still 13.79 % higher than the control. After 20 weeks, the diameter of capillary lumens increased by 9.09 % compared to the previous period and by 24.14 % compared to the control group (Fig. 2B).



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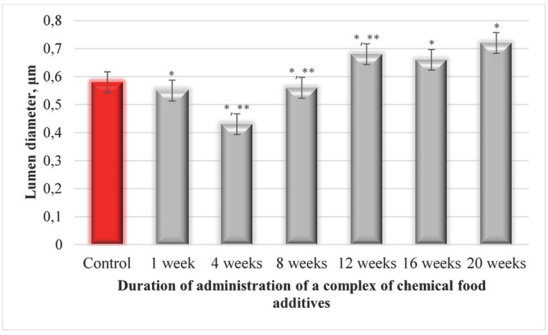

B

Fig. 2. Dynamics of changes in the mean values of the total diameter (A) and lumen diameter (B) of capillaries in the mucosa of the rat ileum at different time points during the administration of monosodium glutamate, sodium nitrite, and Ponceau 4R. Note: * – statistically significant at $p<0.05$ compared to the control group, ** – statistically significant at $p<0.05$ compared to the previous observation period.

Regarding the mean capillary wall thickness, it was determined that after 1 week of administering the experimental solution, this parameter decreased by 29.17 % compared to the control group. After 4 weeks, this trend continued; the morphometric parameter further decreased by 5.88 % compared to the previous period and by 33.33 % compared to the control group ($p<0.05$). After 8 weeks of administration of the complex of chemical food additives, the studied parameter increased by 18.75 % compared to the previous stage but remained 20.83 % lower than the control. After 12 weeks, the mean value of this morphometric parameter decreased by 15.79 % compared to week 8 and was 33.33 % lower than the control ($p<0.05$). At the next stage (after 16 weeks), the parameter increased by 12.5 % compared to the previous period but did not return to control levels, remaining 25 % lower. At the final stage of the study, the mean capillary wall thickness decreased again by 5.56 % compared to the previous period and was 29.17 % lower than the control (Fig. 3).

At the next stage of the study, nitrite concentration in ileum biopsies was measured at various time points following administration of the complex of chemical food additives. After just one week of the experiment, this parameter showed a 100 % increase compared to the control group. After 4 weeks, a 29.41 % decrease was observed compared to the previous value; however, the level remained 41.18 % higher than the control ($p<0.05$). After 8 weeks, the indicator decreased by a further 8.33 % compared to the previous period but was still 29.41 % above the control. A sharp increase in nitrite concentration was recorded at week 12, with a rise of 127.27 % relative to the previous value and 194.12 % compared to the control. Subsequently, at week 16, the indicator increased by a further 2 %, reaching 200 % above the control level. The highest nitrite concentrations were recorded after 20 weeks, representing an increase of 56.86 % compared to the previous period and 370.59 % compared to the control group ($p<0.05$) (Fig. 4).

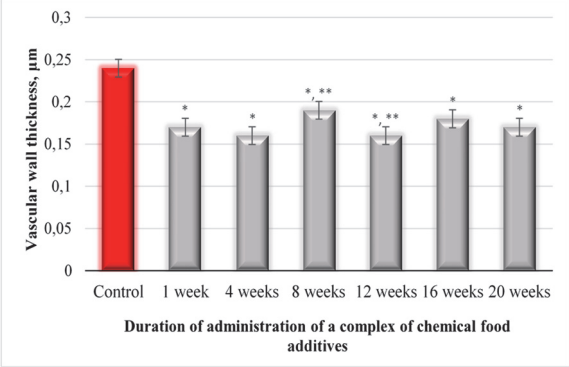

Fig. 3. Dynamics of changes in the mean capillary wall thickness of the ileal mucosa in rats at different time points following administration of monosodium glutamate, sodium nitrite and Ponceau 4R. Note: * – statistically significant at p<0.05 compared to the control group, ** – statistically significant at p<0.05 compared to the previous observation period.

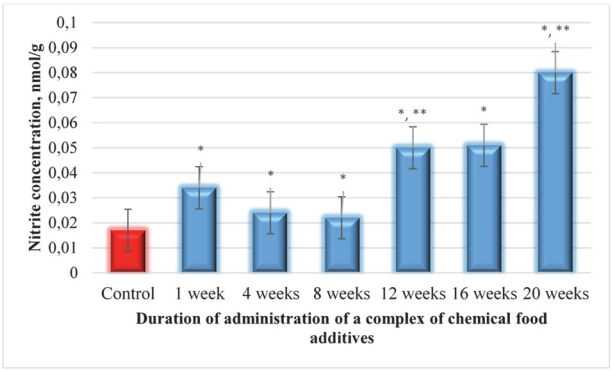

Fig. 4. Dynamics of changes in mean nitrite concentration in rat ileum biopsies at different times of administration of sodium glutamate, sodium nitrite and Ponceau 4R. Note: * – statistically significant at p<0.05 compared to the control group, ** – statistically significant at p<0.05 compared to the previous observation period.

The data obtained indicate a pronounced effect of the combined administration of monosodium glutamate, sodium nitrite and Ponceau 4R on the morphofunctional state of the haemomicrocirculatory bed of the ileal mucosa. A significant decrease in the total diameter of capillaries, their lumen diameter, and capillary wall thickness was recorded as early as 1 week into the experiment compared to the control group. These changes are most likely associated with primary damage to the vascular endothelium, as well as the development of vasoconstriction and dystrophic processes within the vascular wall. The most critical changes were observed at week 4 of the study, characterised by the maximal decrease in all analysed indicators, which may indicate a progressive toxic effect of these food additives and the development of hypoxic changes in the tissues. In the subsequent stages of the experiment (weeks 8–12), partial restoration of capillary blood flow was observed, evidenced by an increase in capillary lumen diameter and vessel diameter, indicating the activation of adaptive-compensatory mechanisms. At the same time, the thickness of the capillary wall showed unstable dynamics, suggesting incomplete repair processes and the presence of long-term chronic damage. The most pronounced compensation was observed at week 16 of the study, when both the diameter and lumen of the capillaries significantly exceeded the control values. Such a response may indicate reactive hyperaemia; however, given the incomplete restoration of the capillary wall structure, its effectiveness remains questionable. At the final stage, a partial decrease in the previously increased indicators was observed, indicating the depletion of compensatory mechanisms or progression of vascular dystrophy. Thus, characteristic signs of increasing degeneration of the walls of the exchange segment vessels were noted, differing from the response of other vessels in the haemomicrocirculatory bed of the ileal mucosa, particularly the capacitive link, where, conversely, wall thickening was observed [5].

These changes closely correspond to the dynamics of increasing nitrite concentration. In ileum biopsies, after just one week of administering the complex of chemical food additives, nitrite concentration doubled compared to the control group, indicating rapid accumulation of compounds or disruption of their metabolism. Despite a slight decrease in the indicator at weeks 4 and 8, the level remained elevated, indicating chronic overload of the detoxification systems. At week 12, a sharp increase in nitrite content was recorded – almost three times higher than in the control group – with a further rise at weeks 16 and 20. This indicates growing accumulation of nitrites in the tissues, which could potentially cause nitrosative stress and damage to the structural elements of the ileal mucosa, particularly its haemomicrocirculatory bed.

Despite the lack of data on changes in the vascular exchange segment of the ileum following administration of monosodium glutamate, sodium nitrite and Ponceau 4R, results from some studies indicate that these structures are quite sensitive to the effects of other exogenous factors [8, 9, 12]. Furthermore, morphological and structural changes in capillaries can lead to dysfunction in various organs and systems [4, 6, 10, 11].

Conclusion

The combined administration of monosodium glutamate, sodium nitrite, and Ponceau 4R exerts a pronounced adverse effect on the structural and functional characteristics of the vessels in the exchange

segment of the haemomicrocirculatory bed of the ileal mucosa, manifested by acute initial damage, unstable reparative processes, and chronic degeneration of the microvascular wall. One of the underlying factors contributing to these alterations is the disruption of nitrite metabolism and the consequent increase in their concentration within the organ. It was established that, already at the early stages of the study, this parameter had doubled compared with the control group and, despite certain downward fluctuations in the medium term, from week 12 onwards progressive nitrite accumulation was observed, persisting until the final stage of the study. The presence of both structural alterations and biochemical deviations provides further confirmation of the damaging impact and underscores the potential risks associated with the prolonged consumption of these chemical food additives.

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