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MORPHOLOGICAL FEATURES OF THE STROMAL COMPONENT OF THE LIVER IN EXPERIMENTAL SUPPLEMENT OF RATIONS WITH FOOD ADDITIVES

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The purpose of this study was to study in an experiment the morphological features of the structural organization of the periportal connective tissue of the liver of white rats after the introduction into the diet of a complex of food additives (sodium glutamate, sodium nitrite, Ponceau 4R) for 8 weeks. The study was performed on 30 outbred white rats of both sexes, weighing 204±0.67 g. The experimental animals were additionally fed a combination of food additives – monosodium glutamate, Ponceau R-4, sodium nitrate for 1 and 8 weeks. The study of the stromal component of the liver was carried out on traditional histological preparations. To differentiate immunocompetent cells of connective tissue structures, immunohistochemical reactions with antibodies to CD68 and CD3 were performed. It was established that the addition of a complex of food additives (sodium glutamate, sodium nitrite, ponzo 4R) to the standard diet of laboratory animals for 8 weeks leads to excessive development of connective tissue in the liver and changes in the relative number of CD3+ and CD68+ cellular elements.

Key words: liver, connective tissue, stroma, food additives, monosodium glutamate, Ponceau R-4, sodium nitrate.

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МОРФОЛОГІЧНІ ОСОБЛИВОСТІ СТРОМАЛЬНОГО КОМПОНЕНТА ПЕЧІНКИ ПРИ ВВЕДЕННІ В РАЦІОН ХАРЧОВИХ ДОБАВОК В ЕКСПЕРИМЕНТІ

Метою цього дослідження було вивчення в експерименті морфологічних особливостей структурної організації перипортальної сполучної тканини печінки білих щурів після введення в харчовий раціон комплексу харчових добавок (глютамату натрію, нітриту натрію, понсо 4R) впродовж 8 тижнів. Дослідження виконано на 30 безпородних білих щурах обох статей, масою 204±0,67г. Експериментальним тваринам додатково вводили до раціону комбінацію харчових добавок – глутамат натрію, Понсо R-4, нітрат натрію протягом 1 і 8 тижнів. Вивчення стромального компонента печінки проводили на традиційних гістологічних препаратах. Для диференціації імунокомпетентних клітин сполучнотканинних структур проводили імуногістохімічні реакції з атитілами до CD68 і CD3. Встановлено, що додавання до стандартного раціону лабораторних тварин комплексу харчових добавок (глютамату натрію, нітриту натрію, понсо 4R) протягом 8 тижнів призводить до надмірного розвитку в печінці сполучної тканини та зміни відносної кількості CD3+ та CD68+ клітинних елементів.

Ключові слова: печінка, сполучна тканина, строма, харчові добавки, глутамат натрію, Понсо R-4, нітрат натрію.

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The liver is actively involved in the metabolism and maintaining the constancy of the internal environment of the body [1]. The blood flowing through the portal vein from the stomach, small and large intestine, pancreas and spleen passes through the liver and is cleared of harmful substances [2]. The morpho-functional state of this organ objectively characterizes the quality of food products that a person uses on daily rations [3]. The diverse food additives are widely used in contemporary cooking, some of which, when taken in significant quantities for a long time, can lead to pathological changes in some internal organs [4–9]. Experimental studies also show a negative effect of the complex use of food additives on hepatocytes [8, 9]. However, the study of the features of the structural organization of the stromal component of the liver in the consumption of products, supplemented with various food additives, was not sufficient. Importantly, the study of the cellular component of the liver stroma and its main elements is relevant, since hepatocytes, sinusoidal cells, leukocytes, connective tissue cells (fibroblasts, mast cells), as well as the extracellular matrix, constantly interact with each other and constitute a single structural and functional system [10]. Stroma cells are involved in the regulation and coordination of both destructive and proliferative processes in the liver during its diffuse damage, which is caused, among other things, by various toxins [11].

The purpose of the study was to provide an assessment of the morphological features of the structural organization of the periportal connective tissue of the liver of albino rats after 8-week long consumption of the complex of food additives (monosodium glutamate, sodium nitrite, Ponceau 4R), supplemented to the daily rations.

Material and Methods. The study involved 30 outbred albino rats, both male and female, weighing 204±0.67 g. The studies were carried out in accordance with the Rules for the humane treatment

of animals in compliance with the requirements of the Declaration of Helsinki of the World Medical Association [12], general ethical principles for working with experimental animals, approved by the National Congress on Bioethics and according to the regulations of the Law of Ukraine No. 3447-IV "On the protection of animals from cruelty" [13].

The animals were assigned into three groups (10 animals in each). Animals of the Group I (intact animals) received standard feed; animals of the Group II and III (experimental groups) received feed, supplemented with the complex of food additives, namely, monosodium glutamate, Ponceau R-4, sodium nitrate for 1 and 8 weeks, respectively.

Upon euthanasia of the animals made under thiopentone anesthesia overdose (at the dose of 200 mg/kg BW), the liver was removed, fragments of which were fixed in 10 % neutral formalin solution for 24 hours. Subsequently, after dehydration, the formalin-fixed material was embedded into liquid paraffin using the "Microm" paraffin blocks filling station by the standard method. Sections with a thickness of 5–7 μm were made from the paraffin blocks on the "Leica" rotary microtome, and stained with hematoxylin and eosin using the conventional technique.

For the differentiation of immunocompetent cells of the connective tissue structures, immunohistochemical reactions were performed with antibodies to CD68 and CD3, with counterstaining of cell nuclei with Mayer's hematoxylin, after which the relative number of CD68+ and CD3+ cellular elements were calculated.

The study of micro-preparations and determination of morphometric parameters was carried out using the Olimpus BX41 microscope, equipped with a digital microphoto attachment and a package of attached licensed software.

Results of the study and their discussion. In the animals of the control group, on histological sections, the stroma of the liver was represented by the strands of fibrous connective tissue of markedly different sizes, surrounding the intrahepatic bile ducts, the branches of the portal vein and the branches of the proper hepatic artery. In the literature, similar connective tissue formations have been called the "portal tracts", "periportal connective tissue". The bile ducts, branches of the portal vein and branches of the proper hepatic artery of the larger and smaller diameter were located in the larger and smaller connective tissue layers, respectively. The findings of the morphometric studies showed that connective tissue occupied 3.25±0.40 % of the liver of intact animals.

Both cellular elements of the blood cells and the fibroblastic cells were found in the periportal connective tissue. The latter were mainly represented by mature fibroblasts, which were characterized by an elongated spindle shape, basophilic cytoplasm, a light ovoid nucleus, with 1–2 nucleoli (fig. 1).

Among the blood cells, lymphocytes were most often found, whereas plasmacytes, macrophages and eosinophilic leukocytes were detected less often. In addition to the described cellular elements, mast cells were visualized quite often in the connective tissue of the portal tracts, near the blood vessels, some of which had signs of degranulation. Blood cells were commonly found in relatively large numbers in large connective tissue strands, surrounding vascular formations of relatively large diameter; fibroblasts were found in slightly greater amount in the small portal tracts.

The findings of the morphometric studies showed that in the connective tissue of the intact animals' liver, fibroblastic cells and blood cells accounted for 47±0.17 % and 53±0.19 %, respectively. At the same time, in the population of the blood cells, CD68+ cells and CD3+ cells accounted for 32.55 % and 47.45 %, respectively.

Following the 1 week of consumption of complex of food additives, supplemented to the rations, a slight increase in the relative amount of connective tissue was observed in the liver of experimental animals, accounting for 4.26±1.10 %.

In the periportal connective tissue, an increase in the number of cellular elements, mainly due to lymphocytes, plasmacytes and macrophage-monocyte cell elements was noted. In the perivascular spaces, mastocytes were found significantly more often compared to the intact animals, and some mastocytes showed morphological signs of degranulation.

The total relative number of the blood cells and fibroblastic cells in the periportal connective tissue accounted for 58±0.26 % and 42±0.29 %, respectively. Among the latter, both immature (less specialized) and differentiated (mature) forms of fibroblasts were detected.

Less specialized fibroblasts were represented by a rather polymorphic cell population, which, apparently, reflected the stages of development and differentiation of the above cellular elements. Notably, quite often we found relatively small orbicular cells, in which the nucleus occupied most of the cytoplasm. At the same time, cellular elements of larger sizes, mostly ovoid in shape, were found, which cytoplasm contained orbicular nuclei, occupying, however, a much smaller volume of the intracellular space.

The ratio in the population of blood cells changed, compared to control animals, where CD68+ cells and CD3+ cells accounted for 27.74 % and 52.26 %, respectively (fig. 2.).

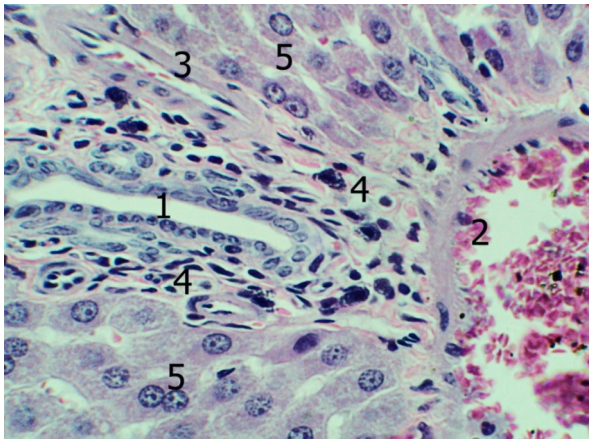

Fig. 1. The structure of the liver of the intact albino rats. H&E stain. Ob. lens: 40× magnification; oc. lens: 10× magnification. 1 – intrahepatic bile duct; 2 – the branch of the proper hepatic artery; 3 – the branch of the portal vein; 4 – periportal connective tissue; 5 – hepatocytes.

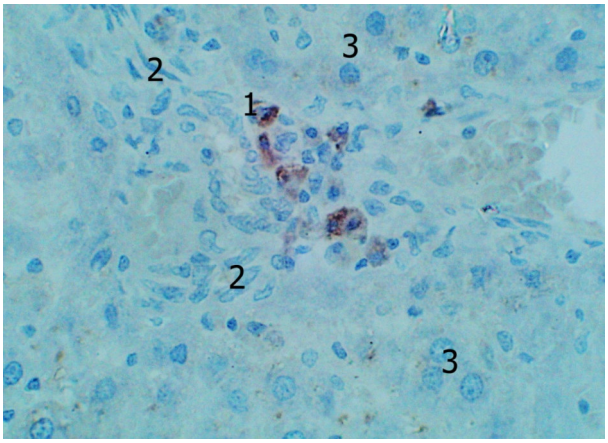

Fig.2. The response of the monoclonal antibodies to CD67 in the periportal connective tissue of the liver of albino rats (combined effect of food additives for 1 week). Mayer's hematoxylin counterstaining. Ob. lens: 40× magnification; oc. lens: 10× magnification.1 – CD67 positive cells; 2 – cellular elements of the fibroblastic cells; 3 – hepatocytes.\

In some observations, macrophage-monocytic cells formed rather large, pronounced focal infiltrates, disseminating beyond the periportal connective tissue (fig. 3.).

Within the 8 weeks of consumption feed, supplemented with the complex of food additives, the relative amount of connective tissue in the liver of laboratory animals increased significantly, compared to the previous experimental group. This indicator was by 2 times greater and accounted for 11.0±2.40 %. At the same time, an increase in the size of the connective tissue fields surrounding the vascular formations, both small and medium caliber, was noted. The described process was pronounced both in the central and peripheral areas of the liver.

In the described connective tissue structures, no increase in the cellular elements was observed. However, some qualitative changes in the cell population of the periportal connective tissue was noteworthy. Thus, the total relative number of lymphocytes, plasmacytes and cellular elements of the macrophage-monocytic cells slightly decreased and accounted for 44±0.25 %, the number of fibroblastic cells, respectively, increased and accounted for 56±0.22 %.

Directly in the population of fibroblastic cells, the relative number of mature fibroblasts increased. Blood cells were found in greater numbers in the connective tissue, surrounding small and medium-sized blood vessels and bile ducts. In the connective tissue fields, surrounding large vascular formations, insignificant amount of blood cells was found, and quite often the latter tended to be focal. The findings of the immunohistochemical studies have established that in the population of blood cells CD68+ cells and CD3+ cells accounted for 47.1 % and 32.92 %, respectively (fig. 4.).

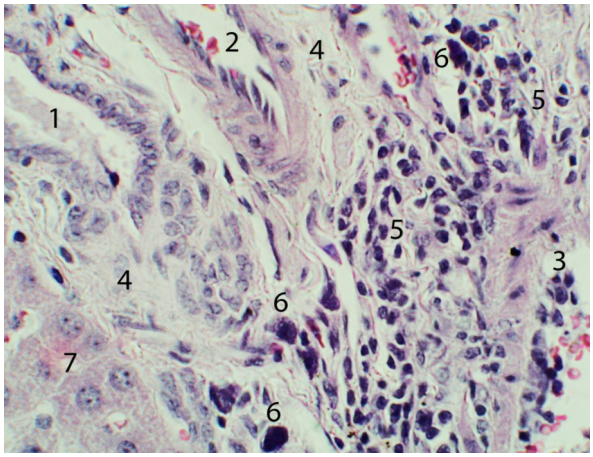

Fig.3. The structure of the liver of albino rats (combined effect of food additives for 1 week). H&E stain. Ob. lens: 40× magnification; oc. lens: 10× magnification. 1 – bile capillary; 2 – the branch of the hepatic artery; 3 – the branch of the hepatic vein; 4 – periportal connective tissue; 5 – lymphoplasmacytic cell infiltration; 6 – mastocytes; 7 – hepatocytes.

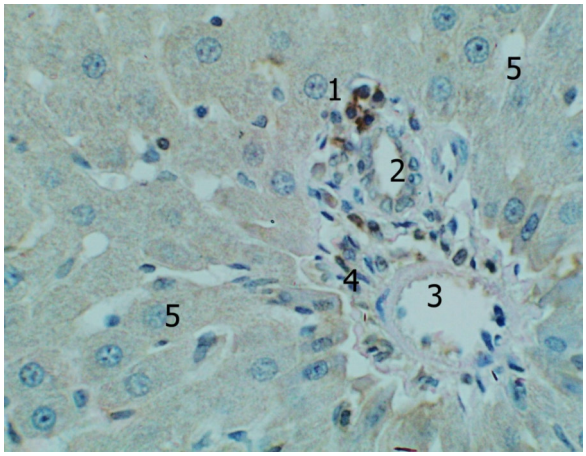

Fig.4. The response of the monoclonal antibodies to CD3 in the periportal connective tissue of albino rats (combined effect of food additives for 1 week). Mayer's hematoxylin counterstaining. Ob. lens: 40× magnification; oc. lens: 10× magnification. 1 – CD3 positive cells; 2 – intrahepatic bile duct; 3 – the branch of the portal vein; 4 – cellular elements of the fibroblastic cells; 5 – hepatocytes.

As before, mastocytes were quite commonly found in the perivascular spaces of the periportal connective tissue, some of which were with degranulation phenomena. As in the previous experimental group, scarce cellular infiltrates localized both in the periportal connective tissue and disseminating beyond its limits to the nearby parenchyma were noted. However, such infiltrates were found less often compared to the previous experimental group, and were smaller in size and consisted mainly of lymphocytes, plasmacytes and small number of macrophages.

The findings of the studies presented above indicate that consumption of the complex of food additives (monosodium glutamate, sodium nitrite, Ponceau 4R), supplemented into the standard rations of the laboratory animals has a significant effect on the stromal component of the liver. First of all, a progressive increase in the relative amount of connective tissue in the liver was notable. This circumstance may be associated with a response to the acute damage of hepatocytes, which occurs in consumption of food additives, supplemented into the rations [9]. The current studies have established that in hepatocytic damage, the synthesis of extracellular stroma components, mainly type I and III collagen, is activated, followed by the development of fibrosis [14, 15]. Consequently, it can be appropriate to suspect the development of liver fibrosis in experimental animals in a longer-term consumption of the food additives.

An increase in the relative number of CD3+ cells in the periportal connective tissue, followed by a significant decrease in the relative number of the latter by the end of the experiment, reflects the dynamics of a nonspecific inflammatory reaction that develops in the connective tissue structures of the liver when food additives are supplemented into the rations. At the same time, throughout the experiment, an increase in the relative number of CD68+ cellular elements were observed.

Conclusion

8-week long consumption of the complex of food additives (monosodium glutamate, sodium nitrite, Ponceau 4R), supplemented to the standard rations of the laboratory animals, leads to excessive development of connective tissue in the liver and a change in the relative number of CD3+ and CD68+ cellular elements.

Prospects for further research will encompass the follow-up study of changes in the liver of rats with longer exposure to food additives in the experiment.

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