ORIGINAL ARTICLE

MORPHOLOGICAL FEATURES OF THE LIVER PARENCHYMA IN THE EXPERIMENTAL SUPPLEMENTATION OF RATION WITH THE FOOD ADDITIVES

DOI: 10.36740/WLek202206117

Haliia M. Mustafina, Ivan I. Starchenko, Boris M. Fylenko, Volodymyr M. Koka, Valentyna V. Cherniak, Nataliia V. Roiko, Serhiy A. Proskurnya
POLTAVA STATE MEDICAL UNIVERSITY, POLTAVA, UKRAINE

ABSTRACT

The aim: The aim of the paper was the experimental study of the morphological features of albino rat hepatocytes after the consumption of the complex of food additives (monosodium glutamate, sodium nitrite, Ponceau 4R) supplemented into the ration and consumed for four weeks.

Materials and methods: The study was performed on 30 outbred albino rats of both genders, weighing 204±0.67 g. The ration of the experimental animals, supplemented with a combination of food additives, namely, monosodium glutamate, Ponceau 4R, sodium nitrate, was consumed for 1 and 4 weeks. The study of the structure of hepathocytes was carried out on traditional histological preparations and preparations stained with Best's carmine.

Results: Supplementation of ration with the complex of food additives for one week showed the phenomena of fatty degeneration that dominated in hepatocytes, and in a longer consumption of food additives in the ration (for four weeks), the number of liver cells with the phenomena of hydropic degeneration significantly increased, while individual hepatocytes had signs of irreversible destructive changes.

Conclusions: Consumption of the complex of food additives supplemented into the standard ration of laboratory animals for 4 weeks leads to a significant change in the dimensions of the liver cells, a decrease in their glycogen content, and a progressive increase in the number of hepatocytes with alterations.

KEY WORDS: liver, hepatocytes, food additives, monosodium glutamate, Ponceau 4R, sodium nitrate

Wiad Lek. 2022;75(6):1525-1528

INTRODUCTION

The liver in the human body plays a key role in the metabolism and, in addition, performs many other vital functions [1]. The main cellular elements of the liver parenchyma are hepatocytes, which are involved in the metabolism of proteins, carbohydrates, cholesterol synthesis, detoxification and excretion of a number of endogenous substances from the body [1,2].

Destructive changes in hepatocytes underlie the pathogenesis of most liver diseases, and therefore, numerous clinical and experimental studies are devoted to the morphofunctional changes of the latter, developed under the influence of various endo- and exogenous factors [3,4]. At the same time, current publications underestimate the description of the structural and functional features of the liver cells when various food additives are supplemented into the ration. At the same time, on the basis of experimental data, it has been found that the complex of food additives (monosodium glutamate, Ponceau 4R, sodium nitrate) supplemented into the ration leads to morphological changes in some organs of the digestive system [5,6].

THE AIM

The experimental study of the morphological features of albino rat hepatocytes after the consumption of the complex of food additives (monosodium glutamate (E621), sodium nitrite (E250), Ponceau 4R (E124)) supplemented into the ration and consumed for four weeks.

MATERIALS AND METHODS

The study involved 30 outbred albino rats of both genders, weighing 204±0.67 g. All experimental studies have been carried out in accordance with the Rules for the Humane Treatment of Animals in accordance with the requirements of the Declaration of Helsinki of the World Medical Association and in accordance with the general ethical principles for working with experimental animals, which were approved by the National Congress of Bioethics [7,8].

The animals were assigned into three groups (10 animals in each). The animals of the first group (intact animals) received regular ration, the animals of the second and third (experimental) groups consumed the combination of food additives, namely, monosodium glutamate, Ponceau 4R, sodium nitrate, supplemented into the ration for 1 and 4 weeks, respectively.

After euthanasia under thiopentone anesthesia overdose (200 mg/kg of the body weight), the liver was removed, the fragments of which were fixed during the 24 hours in the 10% neutral formalin solution. The formalin-fixed mate-

rial, after dehydration, was embedded into liquid paraffin using the "Microm" station for pouring paraffin blocks according to the standard technique. Sections of 5-7 μm thick were made from the paraffin blocks on the "Leica" rotary microtome, which were stained with hematoxylin and eosin according to the conventional technique and Best's carmine to detect glycogen [9,10].

The study of micropreparations and the determination of morphometric parameters were carried out using the Olimpus BX 41 microscope, equipped with a digital microphotographic attachment and a package of attached licensed software.

RESULTS

The findings of the studies have shown that in the liver of albino rats, hepatocytes were the main cellular elements that form the parenchyma of the organ. In typical cases, hepatocytes had distinct contours, a polygonal shape, arranged in two layers, forming hepatic beams, radially diverging from the central vein and separated from each other by intralobular sinusoidal capillaries (Fig. 1). The morphometry has established that the average length of hepatocytes of intact animals was 24.79±1.67 μm; the average width was 17.42±1.11 μm. The average area of the liver cells was 432.5±40.93 μm^2, respectively. The average diameter of the nuclei was 7.61±0.25 μm, the average area of the nuclei was 45.46±3.06 μm^2. The liver cells contained one or two orbicular nuclei. The relative number of binuclear hepatocytes was (20.59±3.16) %. Insufficient number of liver cells with dystrophic changes was found (Fig. 1).

The use of stain for glycogen made it possible to identify three subpopulations of hepatocytes. The most numerous were hepatocytes with a moderate content of glycogen and they generally occupied the intermediate sections of the hepatic lobules; liver cells with a small amount of glycogen granules in the cytoplasm, which were located in the periphery of the hepatic lobules were somewhat less common. The fewest were hepatocytes with a significant content of glycogen and they were localized mainly in the central parts of the hepatic lobules.

The consumption of the complex of food additives supplemented into the ration of the animals for one week showed that the histological structure of the liver did not undergo any significant changes, and there were no noticeable changes in the metric parameters of the liver cells. The average length and width of the latter was 24.26±2.51 μm and 17.07±1.49, respectively. The area of hepatocytes also did not significantly change and accounted for 417.5±78.19 μm^2, respectively. More significant changes were observed in terms of the size parameters of the nuclei, the diameter of which was 8.5±0.28 μm, the average area was 56.6±3.38 μm^2. The relative number of binuclear hepatocytes significantly decreased, compared to the control animals, accounting for (14.53±3.20) %.

In the described experimental group, hepatocytes with dystrophic changes were detected; their total number accounted for 3.7%. At the same time, among the latter, cells with morphological signs of fatty degeneration, which had a dust-like or small-drop appearance, predominated. Changes characteristic of hydropic dystrophy were observed much less frequently in the liver cells.

In addition to the cells described above, we have found liver cells in a small amount with intensely stained, homogeneous cytoplasm, so called "dark hepatocytes". These cells, in addition to the indicated tinctorial features, were characterized by an irregular polygonal shape, variable linear dimensions, an intensely basophilic swollen homogeneous, often pycnotic nucleus. Quite often, such hepatocytes did not have distinct visual contacts with adjacent liver cells, forming hepatic beams. This morphological picture allowed us to consider these cellular elements as hepatocytes, which were at the initial stages of the development of irreversible alterations. Similar cells were found in the subcapsular and in the central portions of the liver and were located mainly on the periphery of the hepatic lobules, near the triads (Fig. 2).

In the animals of the experimental group the number of hepatocytes containing a significant amount of glycogen significantly decreased, compared to the control animals, while the number of liver cells with low glycogen content, on the contrary, increased significantly.

The consumption of the complex of food additives supplemented into the ration of the animals for four week led to significant increase in the size of liver cells, the average length of which was 24.9±1.11 μm, the average width was 19.5±0.87 μm, and the average area, was 484.8±43.08 μm^2, respectively.

The metric characteristics of the nuclei in this group, on the contrary, did not significantly differ from the control values. The average diameter of the nuclei of hepatocytes in this experimental group was 7.8±0.33 μm, the area was 47.8±4.06 μm^2. The relative number of binuclear hepatocytes (15.47±1.52) % was almost the same compared to the previous experimental group.

As previously described, along with hepatocytes with a typical structure, we occasionally detected liver cells with dystrophic changes. The total number of the latter increased significantly and accounted for 8.6% of the entire population of the liver cells. At the same time, the number of hepatocytes with the phenomena of hydropic dystrophy increased markedly, while the number of the liver cells with fatty degeneration, on the contrary, decreased. It is quite possible that a significant number of hepatocytes with the phenomena of hydropic dystrophy along with swelling of the cytoplasm, causes the previously described change in the metric characteristics of liver cells.

In this experimental group, "dark hepatocytes" were also constantly detected, the morphological features of which were described earlier. The number and nature of the localization of the latter in the hepatic parenchyma did not change significantly (Fig. 3.).

Hepatocytes containing a small amount of glycogen were located on the periphery of the lobules, near the hepatic triads, forming the group clusters. Periodically, similar hepatocytes were also found in the intermediate parts of the hepatic lobules, where they tended to be solitary. The total number of described cells slightly increased, compared to the previous group.

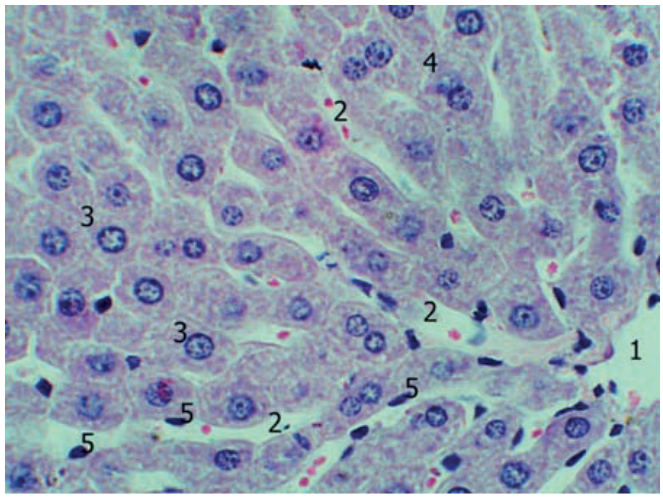

Fig. 1. The structure of the liver of intact albino rats. H&E stain. Objective lens: ×40 magnification, ocular lens: ×10 magnification.
1 – central vein; 2 – intralobular sinusoidal capillaries; 3 – mononuclear hepatocytes; 4 – binuclear hepatocytes; 5 – cells of sinusoid capillaries.

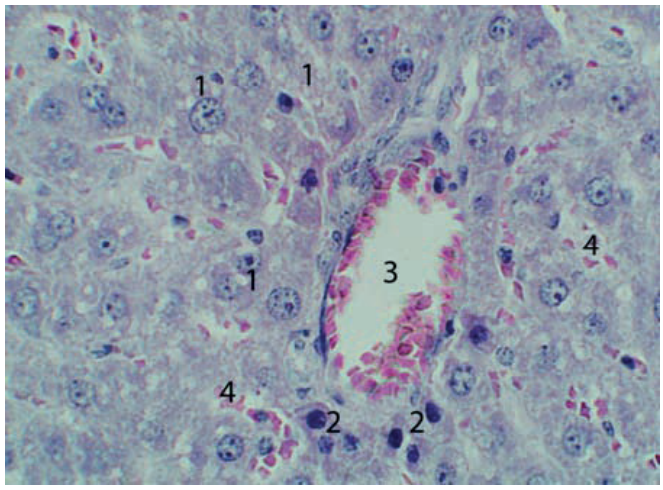

Fig. 2. The structure of the liver of albino rats (combined exposure to the food additives for 1 week). H&E stain. Objective lens: ×40 magnification, ocular lens: ×10 magnification.
1 – hepatocytes with dystrophic changes; 2 – "dark hepatocytes"; 3 – branch of the hepatic vein; 4 – hepatic sinusoids with blood cells

The liver cells containing a moderate amount of glycogen granules occupied mainly the intermediate parts of the hepatic lobules, their number did not significantly change compared to the control animals and the previous experimental group. The number of hepatocytes containing a significant amount of glycogen granules in the cytoplasm significantly decreased; similar to the previous groups they were located in the center of the hepatic lobules.

DISCUSSION

The findings of the study show that the complex of food additives (monosodium glutamate, sodium nitrite, Ponceau 4R) supplemented to standard ration of laboratory animals leads to noticeable morphological changes in the liver parenchyma.

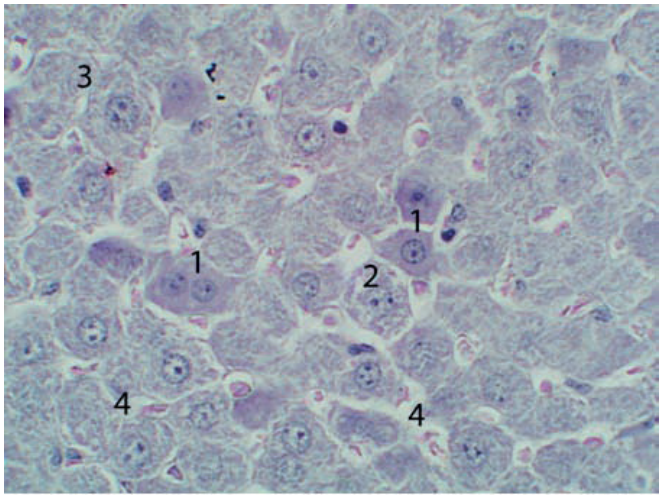

Fig. 3. The structure of the liver of albino rats (combined exposure to the food additives for 4 weeks). H&E stain. Objective lens: ×40 magnification, ocular lens: ×10 magnification.
1 – dark hepatocytes; 2 – hepatocyte with the phenomena of hydropic dystrophy; 3 – enlarged hepatocytes; 4 – hepatic sinusoids with blood cells.

The most significant changes should be considered a change in the metric characteristics of the liver cells, a decrease in their glycogen content and a progressive increase in the number of hepatocytes with alterations. At the same time, consumption of the complex of food additives supplemented into the ration for one week led to the phenomena of fatty degeneration, and a longer term of its consumption (for four weeks) showed that the number of liver cells with morphological features, that are characteristic of hydropic degeneration, noticeably increased. Such changes in the liver occur under the influence of a number of endogenous pathogenic factors, and in some cases may be reversible [11]. The occurrence of "dark hepatocytes" should be assigned to alterations of the liver cells, which, in our opinion, are cells with irreversible destructive changes.

An increase in the size of the liver cells revealed by morphometric studies can be both a consequence of dystrophic changes and a manifestation of the compensatory process associated with an increase in the functional activity of these cellular elements.

CONCLUSIONS

Consumption of the complex of food additives supplemented into the standard ration of laboratory animals for 4 weeks leads to a significant change in the dimensions of the liver cells, a decrease in their glycogen content, and a progressive increase in the number of hepatocytes with alterations.

REFERENCES

1. Mustafina H.M., Starchenko I.I., Koka V.M. et al. Suchasni pohliady na funktsionalnu morfolohiiu ta reparatyvni vlastyvosti pechinky [Modern views on the functional morphology and reparative properties of the liver]. Visnyk problem biolohii i medytsyny. 2020;2(156):43–48. (in Ukrainian)

2. Mustafina H.M. Strukturni osoblyvosti hepatotsytiv bilykh shchuriv [Structural features of white rat hepatocytes]. Aktualni problemy suchasnoi medytsyny: Visnyk Ukrainskoi medychnoi stomatolohichnoi akademii. 2021;21(74):146–150. (in Ukrainian)
3. Mustafina H.M., Starchenko I.I., Koka V.M. Osoblyvosti budovy pechinkovykh klityn za umov tryvaloho vplyvu kharchovykh dobavok v eksperymenti [Peculiarities of life of liver cells for the minds of a thriving infusion of food additives in the experiment]. Suchasni problemy vyvchennia medyko-ekolohichnykh aspektiv zdorovia liudyny: materialy nauk.-prakt. internet-konf. z mizhnar. uchastiu, prysviachenoi 90-y richnytsi z dnia zasnuvannia kafedry medychnoi biolohii v ramkakh sviatkuvannia 100-richchia zasnuvannia Poltavskoho derzhavnoho medychnoho universytetu, Poltava. 2021, 61p. (in Ukrainian)
4. Ferrell L. Liver Pathology: Cirrhosis, Hepatitis, and Primary Liver Tumors. Update and Diagnostic Problems. Mod Pathol. 2000;13: 679–704. doi:10.1038/modpathol.3880119.
5. Mustafina H.M., Starchenko I.I., Koka V.M. et al. Suchasni uiavlennia pro vplyv okremykh kharchovykh dobavok na orhanizm liudyny [Modern ideas about the impact of certain food additives on the human body]. Aktualni problemy suchasnoi medytsyny: Visnyk Ukrainskoi medychnoi stomatolohichnoi akademii. 2021;21(73):194–198. (in Ukrainian)
6. Koka V.M., Starchenko I.I., Roiko N.V. et al. Morfometrychna kharakterystyka miaziv yazyka za umov kombinovanoho vplyvu kharchovykh dobavok v eksperymenti [Morphometric characteristics of the muscles of the tongue under the combined effects of food additives in the experiment]. Aktualni problemy suchasnoi medytsyny: Visnyk Ukrainskoi medychnoi stomatolohichnoi akademii. 2021;21(76):148–152. (in Ukrainian)
7. WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. http://www.wma.net/ [date access 27.11.2021]
8. VII Natsionalnyi konhres z bioetyky [VII National Congress on Bioethics] Natsionalna akademiia medychnykh nauk Ukrainy. http://amnu.gov.ua/vii-naczionalnyj-kongres-z-bioetyky/ [date access 27.11.2021] (in Ukrainian)
9. Bahrii M.M., Dibrova V.A., Popadynets O.H., et al. Metodyky morfolohichnykh doslidzhen [Methods of morphological research]. Vinnytsia: Nova knyha. 2016, 328p. (in Ukrainian)
10. Vareniuk I.M., Dzerzhynskyi M.E. Metody tsyto-histolohichnoi diahnostyky: navchalnyi posibnyk [Methods of cyto-histological diagnosis: a textbook]. Kyiv: Interservis. 2019;256s. (in Ukrainian)
11. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67(1):328–357. doi:10.1002/hep.29367.

The work is a fragment of the research project «Regularities of morphogenesis of organs, tissues and vascular-nervous formations in the norm, in pathology and under the influence of exogenous factors», № state registration 0118U004457.

ORCID and contributionship:
Haliia M. Mustafina: 0000-0002-1596-2486[B-D]
Ivan I. Starchenko: 0000-0002-6666-1448[A,D]
Boris M. Fylenko: 0000-0002-8659-2267[E,F]
Volodymyr M. Koka: 0000-0003-2987-7255[A]
Valentyna V. Cherniak: 0000-0003-4594-565X[C]
Nataliia V. Roiko: 0000-0001-7478-0773[E,F]
Serhiy A. Proskurnya: 0000-0003-2675-9843[E,F]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Haliia M. Mustafina
Poltava State Medical University
23 Shevchenko street, 36011, Poltava, Ukraine
tel: +380503059214
e-mail: margocvt@gmail.com

Received: 11.02.2022
Accepted: 24.05.2022

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article