ISSN 1817-7883 eISSN 2522-9354

ОРИГІНАЛЬНІ ДОСЛІДЖЕННЯ

DOI: 10.31393/reports-vnmedical-2023-27(1)-01
UDC: 616-001.17:615.451.3

HISTOLOGICAL AND HISTOCHEMICAL CHANGES IN LIVER TISSUE OF YOUNG RATS WITH HYPERHOMOCYSTEINEMIA

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Received: December, 23, 2022; Accepted: January, 20, 2023

Annotation. *The decisive place in the synthesis and subsequent metabolism of homocysteine (Hcy) belongs to the liver, where, in particular, transsulfation and remethylation processes take place. It has been proven that organ tissue damage can cause hyperhomocysteinemia (HHcy). Changes in the structural and functional parameters of the liver tissue under the conditions of a significant and long-term increase in blood serum Hcy are still insufficiently studied. Modeling the state of chronic HHcy and studying the features of histological changes in liver tissue at different levels of structural organization is an urgent task. The aim of the research is to study morphological and histochemical changes in the liver tissue of young rats with HHcy. The experiment was carried out on 22 white non-linear young - aged 1-2 months, male rats. During the study, the animals were divided into two groups - control and experimental. Chronic persistent HHcy was modeled by administering thiolactone Hcy in a dose of 200 mg/kg of body weight intragastrically for 60 days to the rats of the experimental group. Histological preparations were studied using a SEO SCAN light microscope and photo-documented using a Vision CCD Camera. Succinate dehydrogenase was detected histochemically according to the Nakhlas method. To study the specifics of glycogen accumulation in hepatocytes, sections were stained using Schiff's reagent, after preliminary treatment with iodic acid (PAS reaction) in the Shabadash modification. It was established that the administration of thiolactone Hcy to young rats at a dose of 200 mg/kg led to impaired blood supply, destructive changes and growth of connective tissue in the liver. Moderate changes in the hepatocyte plates organization, a decrease in the mitotic activity of hepatocytes, and the development of fatty and vacuole-hydropic dystrophy were recorded. Histochemical studies of the liver of animals of the research group established a decrease in the activity of the succinate dehydrogenase enzyme and glycogen content in hepatocytes.*
Keywords: *hyperhomocysteinemia, liver, fatty dystrophy, succinate dehydrogenase, rats.*

Introduction

In modern conditions, the problem of HHcy is highly debatable. Special attention is currently devoted to this topic, since the relationship between the level of sulfhydryl non-proteinogenic amino acid - Hcy in the blood plasma and the appearance and progression of a number of pathological conditions is known for sure. To date, a certain list of somatic diseases has been found, which in one way or another are related to the peculiarities of the metabolism of sulfur-containing amino acids. Among the latter, methionine and the products of its biochemical transformations are of greatest interest. In the course of methionine metabolism, Hcy is synthesized, which, under normal conditions of the body's functioning, undergoes rapid utilization processes. Its norm in blood plasma is considered to be 5-15 μmol/L. HHcy is the result of a violation of the balance between the level of production of Hcy and elimination and is characterized by an increase in the concentration of the specified amino acid more than 100 μmol / l in the case of a severe course of this condition [4, 7, 17].

The decisive place in the synthesis and subsequent metabolism of Hcy belongs to the liver, where, in particular, transsulfation and remethylation processes take place. It has been proven that damage to organ tissue can cause HHcy. In patients with liver cirrhosis, disturbances in Hcy metabolism are also noted. Scientists associate this with inhibiting the synthesis of enzymes, namely methionine synthase, betaine homocysteine methyltransferase, and cystathionine-β-synthase. Non-alcoholic fatty liver disease is relevant in today's conditions, which is becoming more and more common and is usually accompanied by such comorbid pathologies as type II diabetes mellitus, metabolic syndrome, arterial hypertension, and HHcy is a key risk factor for the development of these conditions [1, 5, 9, 13, 15].

Changes in the structural and functional parameters of the liver tissue under the conditions of a significant and long-term increase in blood serum Hcy are still insufficiently studied. At the same time, it is important to establish the actual causes of Hhcy and clearly clarify the order of damage to the organ, since HHcy syndrome occurs not only against the background of morphological changes in hepatocytes, but also due to numerical shifts and even genetically determined conditions (mutations of genes responsible for the synthesis of enzymes of folate metabolism, etc.) [14]. High levels of Hcy in the blood plasma are registered in patients with a deficiency of vitamins B6, B9, B12, excess intake of methionine. In addition, bad habits, a sedentary lifestyle and the use of certain medicines are associated with HHcy [10, 11, 18].

Modeling the state of chronic HHcy and studying the features of histological changes in internal organs, including liver tissue, at different levels of their structural organization is an urgent task. The significant prevalence of liver diseases of unknown etiology forces the search for early markers of them. Since Hcy is one of the factors of a

number of diseases, our study of its influence on the morphology and functional activity of liver tissue is a priority.

The aim of the research is to study the morphological and histochemical changes in the liver tissue of young rats with HHcy.

Materials and methods

The study was carried out in compliance with international recommendations on conducting medical and biological research using animals (Kyiv, Ukraine, 2001) and agreed with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, France, 1986) [6]. The experiment was carried out on 22 white non-linear young - aged 1-2 months, male rats. During the study, the animals were divided into two groups - control and experimental. Chronic persistent HHcy was modeled by administering thiolactone Hcy in a dose of 200 mg/kg of body weight intragastrically for 60 days to the rats of the experimental group. Animals were anesthetized by decapitation using thiopental anesthesia. For histological examination, pieces of liver were taken from pre-weighed animals of all groups. The pieces were fixed in a 10% formalin solution, not exceeding the exposure duration of 1-2 days. Next, the pieces were dehydrated in alcohols of increasing concentration and embedded in paraffin blocks. The prepared sections, 4-5 μm thick, were stained with hematoxylin and eosin and methylene blue [3]. Histological preparations were studied using a SEO SCAN light microscope and photo-documented using a Vision CCD Camera with a system of image output from histological preparations.

To determine the dynamics of compensatory and adaptive processes of the liver under conditions of HHcy, the study of the key enzyme of the tricarboxylic acid cycle - succinate dehydrogenase is of particular interest. This mitochondrial enzyme was detected histochemically by the method of Nakhlas [8]. These studies were carried out on sections made in a cryostat microtome from unfixed tissue using nitro blue tetrazole. The sediment in the form of blue granules of diformazan testified to the presence and localization of the enzyme.

To study the specifics of glycogen accumulation in hepatocytes, sections were stained using Schiff's reagent, after pretreatment with iodic acid (PAS reaction) in Shabadash's modification [8].

The study is a fragment of research works of the Department of Biological and General Chemistry National Pirogov Memorial Medical University , Vinnytsya: "Influence of exogenous and endogenous factors on hydrogen sulfide metabolism and associated metabolic processes in normal and pathology" (state registration number 0113U006461), "The role of exogenous and endogenous sulfur-containing compounds in the mechanisms of internal organs damage and cytoprotection in various pathological conditions" (state registration number 0119U001142).

Results

Microscopic studies of the liver of young rats with hyperhomocysteinemia revealed destructive changes in the organ that developed against the background of impaired blood supply. A slight thickening of perilobular connective tissue was established, its somewhat greater development was observed in the composition of the portal tracts. Some hepatocyte plates were not clearly organized, and the hepatocytes that formed them were polymorphic with varying degrees of development of structural changes in them. Among the hepatocytes there were cells that had normochromic nuclei with well-defined nucleoli, other cells contained hyperchromic nuclei with indistinct nucleoli. Cells with patterns of mitosis in the cytoplasm were also rare. Alterative changes were also manifested in the cytoplasm of hepatocytes, vacuole-hydropic and fatty dystrophy was observed in most cells.

It has been established that hyperhomocysteinemia provokes the development of structural changes in the vascular bed of the liver. Congestion and thrombosis were detected in the central and sublobular veins, as well as in the interlobular veins as part of triads. At the same time, a thickening of their wall was observed in the arteries of the triads. Changes were also reflected in sinusoids. The lumens of most hemocapillaries were expanded, filled with erythrocytes, leukocytes and Kupffer cells. The latter were also found in the perisinusoidal space. Endothelial cells are swollen, Disse's space is expanded, sometimes infiltrated by lymphocytes (Fig. 1).

A histochemical study of succinate dehydrogenase in liver hepatocytes of young rats with simulated hyperhomocysteinemia showed a decrease in the activity of this mitochondrial enzyme in cells. Uneven localization of succinate dehydrogenase in the lobules of the liver was revealed. Large, blue lumps of diformazan stain intensively mainly in hepatocytes on the periphery of the lobule (Fig. 2).

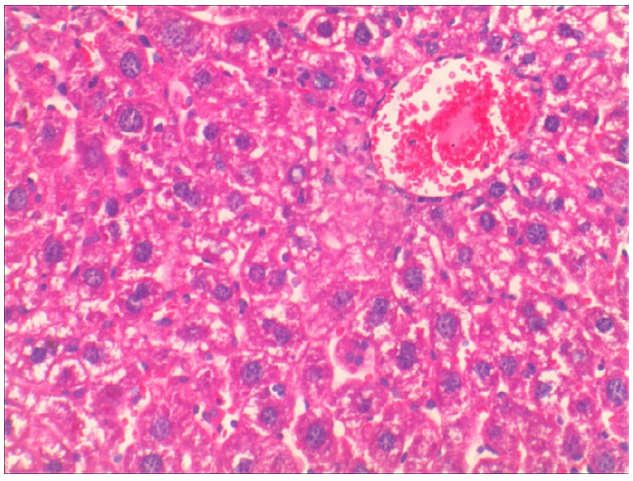

Fig. 1. Histological changes of the liver of young rat under conditions of simulated hyperhomocysteinemia. Dystrophically changed hepatocytes, full-blooded dilated vein. Staining with hematoxylin and eosin. x 200.

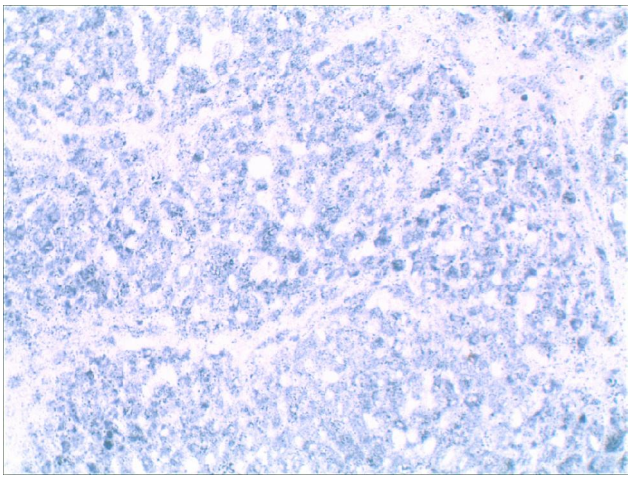

Fig. 2. Low activity of succinate dehydrogenase in the liver of young rat under conditions of simulated hyperhomocysteinemia. Low content of lumps of diformazan in the cytoplasm of hepatocytes. Nakhlas method. x 200.

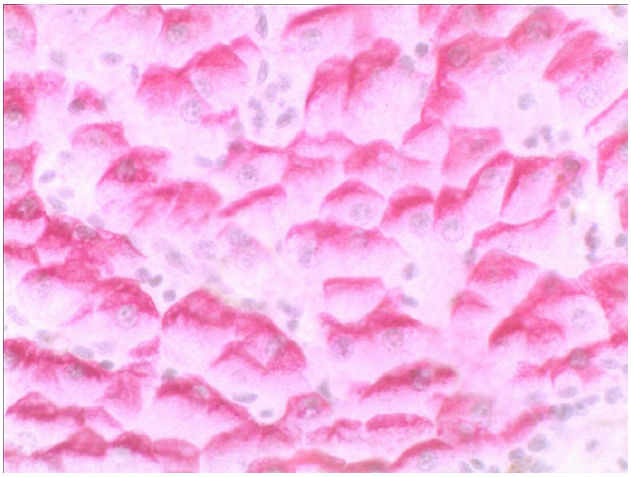

Fig. 3. The presence of glycogen in the liver of a young animal under conditions of simulated hyperhomocysteinemia. A low content of glycogen lumps in the cytoplasm of most hepatocytes. Shabadash method. x 400.

The study of glycogen content in the liver cells of young rats with simulated hyperhomocysteinemia revealed a decrease in this trophic compound. In most cells of the entire lobule, the density of glycogen granules decreases, only in some cells of the peripheral areas of the lobule cells containing many secretory granules are determined (Fig. 3).

Discussion

Researches of Ukrainian and foreign scientists prove the existence of a close relationship between the blood plasma Hcy level and liver pathology. In particular, it was established that Hcy induces the development of fibrosis of the tissue of the organ. The mechanisms of this process are still being studied, but the researchers note that HHcy causes the accumulation of collagen in the liver tissue due to the stimulation of the expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) and, as a result, the accumulation of procollagen I in hepatocytes [12].

Currently, the role of HHcy in the development of hepatic steatosis is being actively investigated. In addition to the known ways of accumulation of lipids in the organ, through experiments on rats, it was shown that a whole complex of nuclear transcription factors and enzymes is involved in the regulation of lipogenesis. Y. Ai et al. (2017) demonstrated that the activity of the transcription factor SREBP1 (sterol regulatory element-binding protein 1) significantly increased in the case of HHcy [2]. The expression of genes dependent on the specified factor encoding enzymes responsible for the synthesis of triglycerides and cholesterol in the liver, namely ACC1α (acetyl-CoA carboxylase 1), HMG-CoAr (β-Hydroxy β-methylglutaryl-CoA reductase), increased in the hepatocytes of experimental animals after injection them Hcy. Scientists also note that one of the possible ways of development of fatty infiltration of liver under these conditions is ER stress, since these organelles are the place of formation of sterols and lipids in the cell. There are hypotheses that under conditions of HHcy in ER, the processes of protein synthesis and their post-translational modification are significantly inhibited due to the inhibition of chaperone activity and the expression of genes regulating lipogenesis is enhanced.

According to studies by M. Stojanovic et al. [16] D, L-Hcy thiolactone when administered to rats contributes to the development of oxidative stress in hepatocytes and the reduction of antioxidant defense reserves. The authors registered inhibition of catalase activity in the liver tissue of laboratory animals. As a result, a significant increase in the H_2O_2 level was observed. HHcy was also associated with increased lipid peroxidation (LP) processes in the organ and accumulation of such product of peroxidation as thiobarbituric acid. LP was accompanied by direct damage of liver cells, their apoptosis, and the initiation of inflammatory processes.

Conclusions and prospects for further development

1. Histological studies of the liver of young rats under HHcy conditions revealed impaired blood supply, destructive changes and growth of connective tissue in the organ. Moderate changes in the hepatocyte plates organization, a decrease in the mitotic activity of hepatocytes, and the development of fatty and vacuole-hydropic dystrophy were recorded. Histochemical studies of the liver of animals of the research group established a decrease in the activity of the succinate dehydrogenase enzyme and glycogen content in hepatocytes.

A promising direction is the significant expansion and deepening of research on the understanding of the development of pathological processes under conditions of hyperhomocysteinemia in the liver of rats of various ages.

ISSN 1817-7883
eISSN 2522-9354
"Вісник Вінницького національного медичного університету", 2023, Т. 27, №1

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ГІСТОЛОГІЧНІ ТА ГІСТОХІМІЧНІ ЗМІНИ В ТКАНИНІ ПЕЧІНКИ МОЛОДИХ ЩУРІВ ПРИ ГІПЕРГОМОЦИСТЕЇНЕМІЇ

Галаган Ю. В.

Анотація. *Визначальне місце в синтезі та подальшому метаболізмі гомоцистеїну (Гц) належить печінці, де відбуваються, зокрема, процеси транссульфування та реметилування. Доведено, що ураження тканини органу може стати причиною гіпергомоцистеїнемії (ГГц). Зміни структурно-функціональних параметрів тканини печінки при умовах значного та довготривалого підвищення в сироватці крові Гц все ще є недостатньо вивченими. Моделювання стану хронічної ГГц та вивчення особливостей гістологічних змін тканини печінки на різних рівнях структурної організації є актуальною задачею. Метою дослідження є вивчення морфологічних і гістохімічних змін у тканині печінки молодих щурів при ГГц. Експеримент проведено на 22 білих нелінійних молодих - віком 1-2 місяці, щурах-самцях. При дослідженні тварин розподіляли на дві групи - контрольну та дослідну. Хронічну стійку ГГц моделювали введенням щурам дослідної групи тіолактону Гц у дозі 200 мг/кг маси тіла інтрагастрально протягом 60 діб. Гістологічні препарати вивчали за допомогою світлового мікроскопа SEO SCAN та фотодокументували за допомогою відеокамери Vision CCD Camera з системою виводу зображення з гістологічних препаратів. Гістохімічно виявляли сукцинатдегідрогеназу за методом Нахласа. Ці дослідження здійснювали на зрізах, виготовлених у мікротом-кріостаті з нефіксованої тканини з використанням нітро-синього тетразолю. Для дослідження особливостей накопичення глікогену в гепатоцитах проводили забарвлення зрізів за допомогою реактиву Шиффа, після попередньої обробки йодною кислотою (PAS-реакція) у модифікації Шабадаша. Було встановлено, що введення тіолактону Гц молодим щурам у дозі 200 мг/кг призвело до порушення кровопостачання, деструктивних змін і розростання сполучної тканини в печінці. Зареєстровано помірні зміни балкової організації, зниження мітотичної активності гепатоцитів, розвиток у них жирової та вакуольно-гідропічної дистрофії. Гістохімічні дослідження печінки тварин дослідної групи встановили зниження активності в гепатоцитах ферменту сукцинатдегідрогенази та вмісту глікогену.*

Ключові слова: *гіпергомоцистеїнемія, печінка, жирова дистрофія, сукцинатдегідрогеназа, щури.*