Morphological changes of the liver under conditions of hyperhomocysteinemia in the background of hypo- and hyperthyroidism

Nechyporuk V.M.1, Korda M.M.2, Kovalchuk O.V.1

1 National Pirogov Memorial Medical University, Vinnytsya, Ukraine
2 I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

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Thyroxine and Triiodothyronine are very important for normal growth, development and organ function. These hormones regulate the basal rate of metabolism of all cells, including hepatocytes, and thus modulate liver function. There is a close connection between hyperhomocysteinemia (HHCy) and the induction of oxidative processes, disruption of nitric oxide production of NO synthase, damage to the endoplasmic reticulum and activation of inflammatory processes in the liver. Disorders of homocysteine metabolism (HC) in thyroid dysfunction are also known. Therefore, it can be assumed that the violation of the structure and functions of the liver will be an important manifestation of the negative impact of HHCy on organs and tissues in hyper- and hypothyroidism.

The aim of the study was to establish the reorganization of the structural components of the liver in the conditions of modelized HHCy, hypo- and hyperthyroidism and their joint effects. Thiolactone HHCy was modelized by administering to animals an exogenous HC in the form of thiolactone at a dose of 100 mg/kg body weight once a day for 28 days. Hyperthyroidism was modelized by daily administration of L-thyroxine at a dose of 200 μg/kg for the 21 days, hypothyroidism - daily administration of thiamazole at a dose of 10 mg/kg for the 21 days. Individual groups of animals were administered L-thyroxine and thiamazole in parallel with HC. It was found that in the conditions of simulated HHCy, hypo- and hyperthyroidism in the liver of experimental animals there is an incompleteness of hepatocyte beams, changes in hepatocytes of destructive, dystrophic and necrotic nature with signs of steatosis, vascular disorders. Conclusions: both HHCy and hypo- or hyperthyroidism lead to a violation of the structural organization of liver tissue. With the development of thyroid dysfunction on the background of HHCy, the disturbances of the histological structure of hepatocytes significantly increased.

Key words: hyperthyroidism, hypothyroidism, hyperhomocysteinemia, homocysteine, liver.

Introduction

Thyroxine and triiodothyronine are necessary for normal growth, development and functioning of organs. These hormones regulate the rate of basic metabolism of all cells, including hepatocytes, and thus modulate liver function; the liver in turn metabolizes thyroid hormones and regulates their systemic endocrine effect. Graves' disease has been shown to cause asymptomatic elevations in liver enzymes, jaundice, and, less commonly, acute liver failure, but the relationship between thyroid hormone levels and liver tissue status remains unclear [18].

Mechanisms associated with disorders of sulfur-containing amino acid metabolism are known to play a significant role in the development of liver pathology. A special role belongs to homocysteine (HC), an intermediate product of methionine metabolism [8]. The accumulation of HC in the blood is a consequence of an imbalance between the level of its synthesis and elimination. A special role in the synthesis and metabolism of the latter is assigned to the liver, in which a significant part of transmethylation reactions occurs [17]. Obviously, elevated levels of HC in the blood can cause liver damage and, conversely, liver damage often leads to disorders of HC metabolism. Cirrhosis of the liver in humans is known to be associated with HC metabolism, in particular the expression of methionine synthase, betaine-
homocysteine-S-methyltransferase, cystathionine-β-synthase genes is reduced [11, 12].

In previous studies, we have shown that the experimental reproduction of hyperthyroidism leads to a decrease in the level of HC, and hypothyroidism, in contrast, causes an increase in the content of HC in the blood, which is associated with changes in the activity of methionine and cysteine [15].

The aim of this study was to establish changes in the structure of the liver under the conditions of HHCy, hyper- and hypothyroidism and their combined effects.

**Materials and methods**

The experiments were performed on 50 outbred white male rats weighing 180-200 g. Rats were kept at standard daylight on a normal diet. All studies were conducted in compliance with the requirements of humane treatment of experimental animals, regulated by the Law of Ukraine “On protection of animals from cruel treatment” (№ 3447-IV of 21.02.2006) and the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, March 18, 1986).

All animals were divided into 5 groups: 1 - intact rats. This group of animals was injected intragastrically with 1% starch solution; 2 - animals with thiolactone HHCy, which was caused by intragastric administration of HC in the form of thiolactone at a dose of 100 mg/kg body weight in 1% starch solution once a day for 28 days. The dose, routes and duration of administration of thiolactone HC are borrowed from the literature and did not cause death of animals [19]; 3 - animals with hyperthyroidism, which were administered intragastrically daily for 21 days L-thyroxine at a dose of 200 µg/kg in 1% starch solution [14]; 4 - animals with thiolactone HHCy, which were daily administered intragastrically for 21 days L-thyroxine at a dose of 200 µg/kg in 1% starch solution; 5 - animals with hypothyroidism, which were daily administered intragastrically for 21 days Mercazolil in 1% starch solution at a dose of 10 mg/kg body weight [14]; 6 - animals with thiolactone HHCy, which were daily administered intragastrically Mercazolil at a dose of 10 mg/kg in 1% starch solution.

Animals were removed from the experiment 24 hours after the last administration of the selected substances. Collection of material for microscopic examinations and its processing was performed according to the generally accepted method [7]. Pieces of liver were fixed in 10% neutral formalin solution, dehydrated in alcohols of increasing concentration, poured into paraffin blocks. The sections made, 4-5 µm thick, were stained with hematoxylin-eosin [7]. Histological specimens were examined using a MIKROmed SEO SCAN light microscope and photodocumented using a Vision CCD Camera with an image output system from histological specimens.

**Results**

Microscopic examination of the liver of white rats of the intact group revealed that the organ has a typical, lobular organization. Stromal loose connective tissue is poorly developed, clearly manifested in the area of triads or portal tracts of the hepatic artery, vein, bile duct, lymphatic vessel and nerves. The lobule is formed by anastomotic hepatic canaliculi consisting of hepatocytes (Fig. 1). Sinusoidal capillaries that flow into the central vein and bile capillaries are located between the canaliculi.

Hepatocytes - the main cells of the organ of irregular polygonal shape have one, sometimes two, weakly basophilic nuclei. The cytoplasm of the cells is little oxyphilic, containing basophilic nodules. The wall of sinusoids is formed by elongated endothelial cells, between which macrophages of the liver, Kupffer cells, are found.

Conducted histological examinations of the liver of animals under the conditions of the simulated HHCy established dyscirculatory disorders with venous plethora, stasis, thrombosis. There is an expansion of lumens and plethora of the central, hepatic and portal veins, and to a lesser extent to the hepatic veins. Large areas of leukocyte infiltrations were identified in the zones of the portal tracts.

Damage to the canaliculi-lobule organization of the organ was revealed. The sinusoidal capillaries are widened, full-blooded, the wall of the capillaries is indistinct. Hepatocytes with swollen cytoplasm and manifestations of vaculo-hydropic and fatty dystrophy were mainly defined in the centrilobular zones. Cells with small-droplet fatty dystrophy were found in certain zones. The nuclei of hepatocytes are hyperchromic, pyknotically altered (Fig. 2). The figures of mitosis in hepatocytes were practically not revealed. The number of Kupffer cells in the wall of sinusoids increased.

In the group of animals with experimental hyperthyroidism, histological changes of the liver are also destructive and manifested by discomplexation of liver canaliculi, expansion and blood supply of sinusoidal hemocapillaries, in their lumens are numerous erythrocytes, leukocytes, and in the wall - activated...
macrophage cells. Vascular disorders are significant, especially plethora is characteristic for central veins, and hepatic portal veins have moderate lumens, but indistinct wall, arteries often have spasmodic lumen, edema and wall thickening, there is significant perivascular edema, histoleukocyte infiltrates (Fig. 3). The number of activated fibroblasts is growing. Hepatocytes are moderately oxyphilic, with signs of cytoplasmic onset, dystrophically altered, containing dark, compacted basophilic nuclei.

Microscopic studies of the liver of experimental animals in the simulated hyperthyroidism and HHCy established a more significant degree of damage to the liver compared to the previous group of observations. The reorganization of the vessels of the organ is manifested by a sharp enlargement, plethora, especially of the central and portal veins, their walls are swollen or vaguely expressed, thinned, which determines the formation of local hemorrhages. The veins in the composition of the triads are also blood-filled, there is a thickening of the wall of the portal arteries. Leukocyte infiltration is defined both in the areas of the potent tracts and along the course of sinusoids (Fig. 4A). Alterative changes in the parenchyma of the organ are manifested by discomplexation of hepatocyte canaliculi. Altered cells with weakly oxyphilic cytoplasm, hyperchromia and pyknosis of the nucleus are mainly observed in the central zones of the lobes. There are signs of hydropic, small- and large-droplet hepatocyte dystrophy, formation of centrilobular necrosis and foci of the lysis (Fig. 4B). Sinusoidal hemocapillaries were mainly determined only in the peripheral areas of the lobules and were filled with blood.

Microscopic studies of the liver under the conditions of a simulated experimental hypothyroidism have established disturbances of the histoarchitectonics of the organ and...
alteration of hepatocytes. Predominantly in the centrilobular zones, the cells were swollen with signs of destruction, vacuolar, small- and large-droplet fatty dystrophy. Most of the nuclei were hyperchromic, pyknotic, but mainly in the peripheral parts of the particle there were normochromic, moderately basophilic nuclei with enlightened weakly basophilic cytoplasm. Most of the vessels are plethora, with large lumens, especially central and portal veins (Fig. 5). There was an uneven blood supply to the sinusoidal hemocapillaries, the full-blown lumens of the microvessels were present mainly on the periphery of the lobules, they were full by erythrocytes, neutrophilic granulocytes, and thrombocytes. Periductal areas are infiltrated by leukocytes, activated fibroblasts.

A microscopic study of the liver of animals under the combined effects of hypothyroidism and HHCy revealed the most pronounced changes in the necrotic and degenerative nature of the structural components of the lobes of the organ on the background of significant vascular disorders. The remodeling of hepatocytes is characterized by small- and large-scale fat dystrophy, and to a lesser extent by protein-vacuolar, as well as step necrosis was also found in the lobes of the liver (Fig. 6B). The heteromorphism of the cells in the composition of the cell is observed, they were present as "light" and "dark" hepatocytes, which were in a state of functional tension. Inflammatory changes in the organ were manifested by large leukocyte infiltrates, both in the zone of the portal tracts and around the central veins and bright areas of the lysis in the areas of destroyed hepatocytes (Fig. 6A).

**Discussion**

The results of microscopic examination of the liver at HHCy are consistent with the available data from the literature. High levels of HC in plasma are associated with the development of hydropic fatty liver disease. In the work of D.O. Nekrut et al., 2017 found that HHCy causes disorders of biosynthetic processes in hepatocytes and is characterized by the development of small droplet fat dystrophy [16]. It was also established that HHCy causes an increase in the number of Kupffer cells in the sinusoid wall. In addition, there is damage to the microcirculation of the liver and the development of fibrogenesis [3]. It is known that one of the reasons for the development and progression of non-alcoholic fatty liver disease may be high levels of HC in the blood. HHCy is one of the important causes of steatohepatitis, changes in the lipid spectrum of the blood, and subsequently - the development of the process into fibrosis and cirrhosis of the liver [5]. HHCy also has a toxic effect on the endothelium of hepatic vessels due to the formation of significant amounts of free radicals and the development of endoplasmic reticulum stress [9]. We found a negative effect of HC on protein, carbohydrate and fat metabolism in liver cells, which at the optical level is manifested in the form of steatosis, multilobule fibrosis with signs of parenchymal and stromal reactions [1].

**Fig. 5.** Histological changes of the animal liver under the conditions of experimental hypothyroidism. Blood-filled central vein, destructively altered hepatocytes, full-blooded sinusoidal capillaries. Hematoxylin-eosin. x200.

**Fig. 6.** Histological changes of the animal liver in modeled hypothyroidism and hyperhomocysteinemia. A - Vessels of the portal tract with paravasal and periductal leukocyte infiltration, dystrophically altered hepatocytes. Hematoxylin-eosin. x200. B - Hepatocytes with small and large droplet fatty dystrophy, areas of cell lysis. Hematoxylin-eosin. x200.
Hyperthyroidism had a destructive effect on liver tissues, namely, we found discomplexation of hepatic canaliculi, enlargement and plethora of sinusoidal hemocapillary cells (numerous erythrocytes, leukocytes in the lumen, Kupffer cells activated in the wall). In study [4], the relationship between hyperkinetic circulation, hypermetabolism and hyperactivity of the sympathetic nervous system in hyperthyroidism and liver damage in cirrhosis was also noted. The authors found disorders of the vascular system (plethora of the central veins), spasmodic lumen of the arteries, swelling and thickening of the vascular wall, significant histoleukocyte infiltrates. The number of activated fibroblasts increased, hepatocytes were moderately oxyphilic (there was an edema of cytoplasm, dystrophically altered, contained compacted dark basophilic nuclei). Recent studies have shown that liver dysfunction can occur in hyperthyroidism as a result of the use of antithyroid drugs. The authors found that patients with Graves’ disease are prone to acute hepatitis, which complicates treatment with antithyroid drugs [2].

We found that in hyperthyroidism and HHCy there is a greater degree of damage to the structural components of the liver in comparison with animals with hypothyroidism. In this case, the vessels of the liver were with a sharp dilation, plethora, especially the central and hepatic portal veins, the walls of the vessels were thinned, swollen, which caused the formation of local bleedings. Blood-filled veins were found in the composition of the triad and thickening of the wall of hepatic arteria, leukocyte infiltration both in the areas of the portal tracts and along the way of sinusoids. Against the background of hyperthyroidism, alternative changes are observed in the liver parenchyma, which was manifested by discomplexation of hepatocyte canaliculi. Altered cells with weakly oxyphilic cytoplasm, hyperchromia and pyknosis of the nucleus are mainly observed in the central zones of the lobes. Signs of hydropic, small- and large-droplet hepatocyte dystrophy, formation of centrilobular necrosis and loci of the lysis are determined. It is possible that high levels of thyroxine, increasing oxygen consumption, increases the amount of free radicals in the liver and, thus, causes damage to liver cells [13]. In another study, it was found that hyperthyroidism activates both the oxidative and antioxidant systems in brain, liver and heart tissues [6]. The authors of this study in rats suggested that the treatment of hyperthyroidism may inhibit the development of cirrhosis, which reflects the direct effect of hyperproduction of thyroid hormones on the liver. We found that experimental hypothyroidism caused destructive histological changes of the liver, which was manifested by discomplexation of liver canaliculi, expansion and full-blood of sinusoidal hemocapillaries with numerous erythrocytes, leukocytes in the lumens, and in the wall were found Kupffer cells. There were significant vascular disorders, blood supply disorders, especially characteristic for central veins, and hepatic portal veins had moderate lumens, arteries often had spasmodic lumen, edema and wall thickening, there is significant perivascular edema, histoleukocyte infiltrates. A significant number of activated fibroblasts and moderately oxyphilic dystrophically altered hepatocytes with signs of cytoplasmic edema containing dark, compacted basophilic nuclei were found. Similar results were also obtained by Makarova N. G. and etc. [10], who observed centrolobular foci of necrosis in hepatocytes in hypothyroidism. The authors did not detect dystrophically altered hepatocytes, which can probably be explained by the rapid transition from dystrophic to necrotic. The authors concluded that the increase in the mass of Kupffer cells is associated with high acid phosphatase activity, as evidenced by the strengthening of the phagocytic function of the liver macrophages, the elimination of necrotic mass. Despite the destructive processes, the content of glycogen in hepatocytes did not change, and the content of total protein increased. The authors explain such changes by compensatory activation of intralobular blood flow, as evidenced by the expansion of sinusoidal capillaries and an increase in their mass by 1.5 times.

The combined effect of hypothyroidism and HHCy causes the most pronounced changes in the necrotic and degenerative characteristics of the structural components of the liver lobes against the background of significant vascular violations. Small and large-scale fatty dystrophy and, to a lesser extent, protein-vacuolar and liquid necrosis in the lobes of the liver were detected. It is obvious that HHCy causes toxic effects on the endothelium of hepatic vessels due to the production of significant amounts of free radicals and stress of the endoplasmic reticulum. The heteropomorphism of cells and inflammatory changes in the organ, which appeared by large leukocyte infiltrates, both in the zone of the portal tracts and around the central vein, were established.

**Conclusions**

Under the conditions of simulated HHCy, hypo- and hyperthyroidism and especially with their combined effect in the liver, significant alternative and dystrophic changes of structural components are observed, characterized by the development of steatosis, vascular disorders, discomplexation of liver canaliculi in the lobules, necrotic changes in hepatocytes.

**References**


Key words: гіпергомоцистеїнемія, гомоцистеїн, печінка, гіпотиреоз, гіпертиреоз, гіпергомоцистеїнемія, гомоцистеїн, печінка.
оксида азота NO-синтазой, повреждением эндоплазматического ретикулума и активацией воспалительных процессов в печени. Известно также о расстройствах метаболизма гомоцистеина (ГЦ) при дисфункции щитовидной железы. Поэтому, можно предположить, что нарушение структуры и функции печени будет важным проявлением негативного влияния ГЦ на органы и ткани при гипер- и гипотиреозе. Целью работы было установление реорганизации структурных компонентов печени в условиях смоделированной ГЦ, гипер- и гипотиреоза и их совместном влиянии. Тиолактоновую ГЦ моделировали введением животным экзогенного ГЦ в виде тиолактона в дозе 100 мг/кг массы тела один раз в сутки в течение 28 суток. Гипертireоз моделировали путем ежедневного введения L-тироксина в дозе 200 мкг/кг в течение 21 дня, гипотиреоз - ежедневного введения мерказолила в дозе 10 мг/кг в течение 21 дня. Отдельным группам животных вводили L-тироксин и мерказолил параллельно с ГЦ. Установлено, что в условиях смоделированной ГЦ, гипо- и гипертиреоза в печени экспериментальных животных наблюдаются дискомплексация балок гепатоцитов, изменение гепатоцитов деструктивного, дистрофического и некротического характера с признаками развития стеатоза, сосудистые расстройства, проявляющиеся неравномерным кровенаполнением, стазами, паравазальнymi лейкоцитарными инфильтратами, мелкими кровоизлияниями. Таким образом, как ГЦ, так и гипо- или гипертиреоз приводят к нарушению структурной организации ткани печени. При развитии дисфункции щитовидной железы на фоне ГЦ нарушение гистологической структуры гепатоцитов существенно усиливалось.

Ключевые слова: гипертиреоз, гипотиреоз, гипергомоцистеинемия, гомоцистеин, печень.