Original paper

Ultrastructural liver changes in the experimental thyrotoxicosis

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Aim of the study is to evaluate ultrastructural changes of rat liver in experimental thyrotoxicosis.

For the study, 36 male rats have been utilized, weighing approximately 150-190 g, which were divided into three groups: the first, control group (12 animals) was composed of healthy rats that received intragastric sodium chloride 0.9% solution, the second group (12 animals) – animals with experimental thyrotoxicosis, which received intragastric solution of L-thyroxine at the rate of 200 μg/kg for 2 weeks, and the third group (12 animals) – rats with experimental thyrotoxicosis, which received intragastric solution of L-thyroxine at the rate of 200 μg/kg for 4 weeks. For electron-microscopic studies small pieces of liver tissue were taken at the end of the 2nd and 4th weeks of the experiment. The material was studied and documented in electron micrographs by using a TEM-125K electron microscope.

In experiment in white male rats the electron-microscopic state of the liver in thyrotoxicosis has been studied. It has been established that thyrotoxicosis is accompanied by the significant changes of the hepatocytes ultrastructure, blood and bile capillaries. Experimental thyrotoxicosis causes significant damage of the liver plasma membranes and intracellular structural components of hepatocytes and endothelial cells.

In experimental thyrotoxicosis, on the background of microcirculatory disorders, significant damage of plasmatic and intracellular organoid membranes of hepatocytes in the liver develops, which has an adverse effect on the functionality of the organ. The found ultrastructural changes are aggravated depending on the duration of thyrotoxicosis.

Key words: thyrotoxicosis, liver, ultrastructural changes, rat.

Introduction

Thyroxine (T4) and triiodothyronine (T3) are essential for normal organ growth, development, and function. These hormones regulate the basal metabolic rate of all cells, including hepatocytes, and thereby modulate hepatic function [1].

A close relationship between thyroid disease and the morphological-functional condition of the liver can be tracked. The liver, consequently, plays an important role in the metabolism of thyroid hormones: it is involved in de-iodisation of thyroid hormones with the formation of their active and inactivated forms [2, 3, 4], in the conversion of organic iodine

into iodide; a number of plasma proteins that bind the lipophilic thyroid hormones are synthesised in the liver [1]. Abnormal liver function is observed in 15-76% of patients with pathology of the thyroid gland, according to different authors [1, 5, 6].

The liver abnormalities such as changes in alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin levels, which can be found in the case of thyrotoxicosis, are thought to be induced by the metabolic effects of thyroid hormone excess and hepatic tissue hypoxia, which occur as a result of enhanced splanchnic oxygen consumption and an increase in the hepatic requirement for oxygen [7, 8].

Therefore, considering the functional relation of liver and thyroid gland and the small number of scientific papers devoted to the study of liver structure in thyrotoxicosis, investigation of the peculiarities of ultrastructural changes of hepatocytes in the simulation of thyrotoxicosis is topical.

Material and methods

This study utilised 36 male rats, weighing approximately 150-190 g, which were divided into three groups: the first, control group (12 animals) was composed of healthy rats that received intragastric sodium chloride 0.9% solution in a volume of 0.5 ml with the help of a catheter. The second group (12 animals) – animals with experimental thyrotoxicosis, which by means of the catheter received intragastric aqueous solution of L-thyroxine at the rate of 200 μg/kg for 2 weeks, the third group (12 animals) – rats with experimental thyrotoxicosis, which received intragastric aqueous solution of L-thyroxine at the rate of 200 μg/kg for 4 weeks.

The development of thyrotoxicosis occurred within 14 days. Animals of the third group had all the signs of thyrotoxicosis by the end of the experiment. The indicator of the condition of thyrotoxicosis was based on the increase of rectal temperature up to 40 $\pm$0.2°C and increased levels of T4 in comparison with control. Indices of T4 were determined by ELISA on day 14 in the blood of rats taken by blood sampling from the cardiac chambers (Table I). Serum samples were evaluated for quantitative determination of free T4 concentration by an electrochemiluminescence immunoassay "ECLIA" and competitive enzyme immunoassay (Elecsys FT4 II Gen, Roche Diagnostics, Germany). All parameters were measured according to the manufacturer's instruction by commercial kits (Roche Diagnostics). In addition, the signs of attaining the state of thyrotoxicosis were also the typical visual symptoms of thyrotoxicosis – hair loss, decrease of body weight, and altered behavioural responses (animals stopped cleaning their fur, became languid).

The animals were kept and manipulated in accordance with the provisions of the law of Ukraine "On protection of animals from brutal treatment" (№ 1759-VI on 15.12.2009). The material sampling was performed on the 14th day in the first (6 animals) and second groups from the beginning of the experiment and on the 28th day in the first (6 animals) and third groups. Thiopental sodium (25 mg/kg) was used for intraperitoneal anaesthesia of the animals before sampling the material.

For electronic-microscopic studies, small pieces of liver tissue of cubic shape and size 1 mm^3 were taken, then they were fixed in 2.5% glutaraldehyde solution, and post-fixed by 1% solution of osmium tetroxide in 0.1 M phosphate buffer (pH 7.2-7.4). Further processing was carried out according to the standard technique [9]. Ultra-thin sections were prepared on ultramicrotome UMTP-7, first contrasted in 2% solution of uranyl acetate, and then in lead citrate solution by Reynolds's method. The material was studied and documented in electron micrographs by using a TEM-125K electronic microscope.

Results

Electronic-microscopic analyses of liver in rats of the second group revealed a significant blood supply in sinusoids, in many cases the spaces of Disse were poorly expressed (Fig. 1).

Hepatocytes with the enlightened cytosol were observed in different areas of hepatic lobules. The cells were limited by plasmalemmas, which on separate areas were not clearly contoured and were locally thickened. Granular and agranular endoplasmic reticula were partially destroyed, the tubules were fragmented, the structural components of Golgi complex were poorly expressed, compared with well-developed tubules of the granular endoplasmic reticulum and the tubules and sacs of smooth endoplasmic reticulum, vacuoles and vesicles of Golgi complex in hepatocytes of intact animals. Round-oval mitochondria of many cells had homogenised matrix and reduced cristas. The granules of glycogen were single, and the amount of lysosomes was increased (Fig. 2).

Table I. Dynamic of free T4 in male rats before the experiment and after 14 days (Mean ± SD)

Animal groups	Control group (n = 12)	Experimental thyrotoxicosis (n = 12)
FT4, day 0, nmol/ml	7,96 ±0,198	7,89 ±0,215
FT4, day 14, nmol/ml	8,43 ±0,202	15,14 ±2,64*

** statistically significant ($p < 0.05$) group with experimental thyrotoxicosis vs. control group*

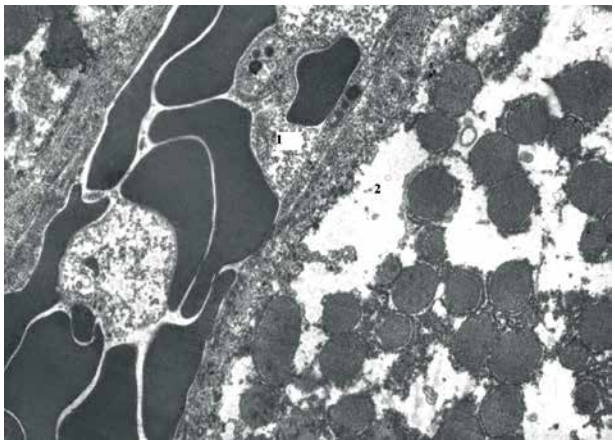

Fig. 1. The hepatic sinusoid fragment after 2 weeks of the experiment. Wide lumen with erythrocytes (1), the enlightened cytoplasm of endothelial cells (2), solitary microvilli in the space of Disse (3) (magnification of × 17K)

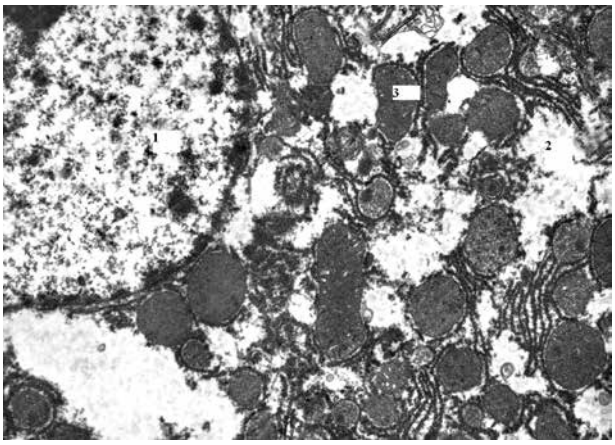

Fig. 2. Ultrastructural imaging of the hepatocyte. The liver after 2 weeks of the experiment (rat). Round nucleus (1), the enlightened area of the cytoplasm (2), mitochondrial destruction (3) (magnification of × 17K)

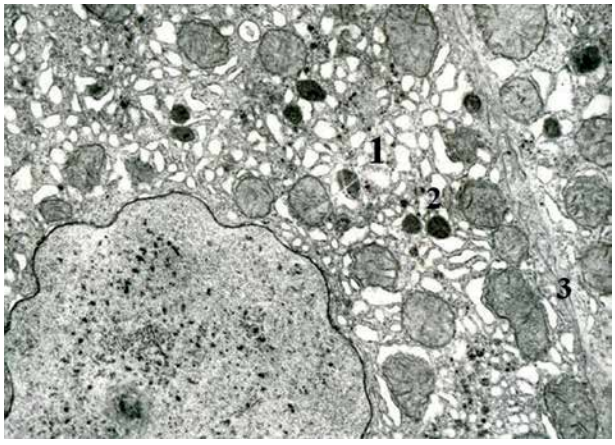

Fig. 3. Hepatocyte submicroscopic changes. The liver after 4 weeks of the experiment (rat). Vacuolated and fragmented cisternae of Golgi complex (1), lysosomes (2), unclear contours of the plasmalemma (3) (magnification of × 21K)

In biliary zones of hepatocytes dilated bile capillaries with a small number of microvilli were observed, and some of such structures contained collapsed lumens.

Four weeks after the beginning of the modelling of thyrotoxicosis in the third group of animals, submicroscopic studies found more significant changes in the liver of experimental animals. The expansion and hyperaemia of sinusoid capillaries was preserved. Besides the hepatocytes with light karyo- and cytoplasm, dark cells cytosol with increased osmophilia were also present. In hepatocytes, there were significant changes in nuclei and cytoplasm: the nuclear shell was wavy, had invagination, perinuclear spaces were locally increased, the density of the nucleolus changes, the tubules of granular endoplasmic reticulum, and cisternae of the Golgi complex were fragmented and unevenly thickened (Fig. 3).

Electronic-microscopic studies indicated increasing changes in plasmatic, organoid, and nuclear membranes. A pronounced fragmentation and vacuolisation of the endoplasmic reticulum and Golgi complex developed, and many of the bile capillaries were widened and had no microvilli. In hepatocytes, the heterogeneity of mitochondria was detected, and the size of the part of these organelles increased, leading to an increased electron density of the matrix and cristae destruction. There were even changes in their external membrane, it became wavy and obscure. The number of lysosomes significantly increased; there were autophagosomes in the cytoplasm of hepatocytes. Naturally, the amount of peroxisomes decreased with a simultaneous increase in their size.

Discussion

As a result of electron-microscopic studies, damage of plasma and intracellular membranes of structural components of hepatocytes and endothelial cells as well as the signs of cholestasis were detected. The spaces of Disse in damaged areas of the hepatic lobules were enlarged, microvilli in their lumens were partially or completely destroyed. The granular and agranular endoplasmic reticulum destruction, the fragmentation of tubules, and a decrease in the content of ribosomes and polisomes were observed. This condition of organelles indicated a decrease in synthetic processes.

An increase of the lysosomes content, decrease of the villi number in the biliary capillaries points to the evidence of degenerative processes in hepatocytes was seen. The presence of hepatocytes with irregularly enlightened cytosol due to organelles damage indicated low functional ability of cells. The energy supply of cells was also violated, shown by hypertrophy of mitochondria with destruction of cristae, and decrease of the glycogen content in the cytoplasm. The emergence of "megamitochondria" due to pathological

conditions is evidence of reparative processes inside the cell, although such structural changes are also the result of a decrease in respiratory activity. Detection of giant mitochondria in liver biopsy at its alcoholic affection indicates a "soft" form of the disease and its more favourable course [10].

The changes mentioned above are not specific and defined during the modelling of ultraviolet C radiation, intestinal obstruction, peritonitis, and the aging process that is caused by a possible common pathogenetic section of these pathological processes. Dystrophic and destructive changes in hepatocytes and haemocapillaries of the sinusoidal type at the abovementioned pathology are manifested by almost similar changes, which include the following: marked swelling of the cellular matrix, local mitochondria and smooth endoplasmic reticulum destruction, partial or complete reduction of the microvilli of the bile capillaries and spaces of Disse, the accumulation of heterochromatin on the periphery of the nucleus, and areas of nuclear membrane destruction [11, 12, 13]. A universal mechanism is common to the pathological conditions mentioned above: the progression of the endogenous intoxication manifestations, activation of lipid peroxidation, the reduction of the antioxidant defence activity, and immune system disorders. Furthermore, adding drugs with antioxidant properties to the basic treatment led to a significant correction of negative changes in the liver at the ultrastructural level [13].

However, if patients with Graves' disease undergo liver biopsy, histological changes are less expressed than in experimental studies. In the liver, with electron-microscopic examination, hyperplasia of the smooth endoplasmic reticulum is revealed, as well as the reduction of the cytoplasmic glycogen and increase of mitochondria size, which contain an excessive amount of cristae [14].

The experiment established that an excess of thyroid hormones can have a direct toxic effect on the liver. Due to this data, a direct effect of T3 excess is implemented by activating a caspase cascade and leads to cells apoptosis. However, the researchers do not exclude the possibility of liver affection due to secondary systemic effect of thyroid hormones excess [15].

Conclusions

The investigation shows that in experimental thyrotoxicosis in the liver, on the background of microcirculatory disorders, a significant damage of plasmatic and intracellular membranes of hepatocytes develops. Destabilisation and destruction of the plasmatic and organoid membranes have an adverse effect on the functionality of the organ. The established ultrastructural changes are aggravated depending on the duration of thyrotoxicosis.

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The authors declare no conflict of interests.

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