ELECTRICAL CONDUCTIVITY OF LIVER TISSUE AS AN INDICATOR OF STRUCTURAL AND FUNCTIONAL ORGANIZATION OF LIVER TISSUE

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Summary. We investigated the possible interrelation between the morphological features of the liver tissue and its electrical conductivity in order to develop a rapid method for analyzing the state of the liver. To do this, we used intact rat liver tissue, the liver at the initial stages of Cu-induced liver fibrosis, and the influence of two hepatoprotective substances – the mix factor and chlorogenic acid. Morphological changes and electrical conductivity of the studied samples were determined. It was found that a change or violation of the structural organization of the liver tissue affects electrical conductivity. We came to the conclusion that electrical conductivity can be used in studies of the structural organization of tissue.

Key words: Cu-induced liver fibrosis, conductivity of liver tissue, liver histopathologies, chlorogenic acid, mix factor

Introduction. The liver is known to be one of the central organs of metabolic regulation, as it provides both synthesis of a significant number of macromolecules and modification, decomposition and detoxification of a lot of metabolites and xenobiotics. Disruption of the balance between these processes in the liver is usually accompanied by changes in its structural and functional characteristics, and in case of long-term deviations from the homeostatic norm, pathological processes take place. Pathological changes in the liver can be induced by extremely various biological (viruses, bacteria), physical and chemical factors.

Pathological processes in the liver, independent of the pathogenetic factor, go through a number of consistent stages: hepatitis, fibrosis, cirrhosis and/or carcinogenesis. Currently, mortality from liver diseases ranks 5th in the world [[1]]. The study of the mechanisms of formation and development of liver fibrosis is the basis for the development of ways to prevent and treat liver diseases. Such fundamental studies can be carried out only on experimental animal models. Although there is an intensive search for modern methods of evaluating the
functional state of the liver, morphological studies are still the gold standard in hepatological diagnostics. However, establishing a diagnosis at early or initial stages, significant changes in structural and functional characteristics that may lead to fibrosis in the future is very difficult, and most often impossible. It is connected with the fact that this functionally important organ is always influenced by a variety of endo- and exogenous factors and their combination, and even in seemed normal conditions there are adaptive structural and functional rearrangements of the liver tissue [[2]]. In this regard, the development of additional methods for assessing the structural and functional states of liver tissue is an urgent biomedical task. When solving this problem, we proceeded from the fact that the assessment method should be fast and allow us to measure the integrative structural-functional state of liver tissue.

Earlier in a series of works [[3]] it was shown that electrical conductivity in biological systems, which are characterised by complex dynamic and multilevel structural organisation, depends not only on the number of ions and charged molecules moving in the electric field, but also on the structural organisation features, including quantitative and qualitative organisation of membranes and connective-tissue formations. The study of structural and functional changes in the liver at the initial stages of Cu-induced fibrosis development showed that the most pronounced changes were observed in the thickening of Glisson’s capsule, partial autolysis of membranes [[4]]. It could be expected that these structural changes in the liver could affect the electrical conductivity of these tissues. It cannot be excluded that at the initial stages of fibrosis development in the liver, changes in ionic composition cannot also occur, in particular in the case of Cu-induced fibrosis. In studies of our laboratory it was shown that biologically active substance mix factor can eliminate biochemical manifestations of liver fibrosis [[5],[6]]. Active research on the hepatotropic activity of chlorogenic acid is ongoing [[7]].

Assessment of the electrical conductivity of liver tissue at the initial stages of Cu-induced liver fibrosis development and its possible changes after administration of such hepatotropic substances as mix factor and chlorogenic acid to animals with liver fibrosis may allow us to evaluate such integrative characteristics of the liver as changes in its structure and ionic composition. The solution of such a problem is relevant not only from the perspective of diagnosis, but also possible mechanisms of development of this pathology.

Methods. Experiments were performed on adult mature males of the Wistar line. 20 animals were divided into 4 experimental groups: 1 - control group in which fibrosis was not induced, 2 - experimental group with Cu-induced fibrosis, 3 - group with liver fibrosis that received mix factor and 4 - group with liver fibrosis that received chlorogenic acid. Cu-induced fibrosis was obtained as described [[4]]. Mix factor was administered per os at a dose of 0.05 ml / 100 g of body weight and chlorogenic acid at a dose of 20 mg / 100 g of body weight. All accepted bioethical rules were followed when working with animals (protocol of the bioethical committee meeting No. 1 dated 03.03.2023). After removal of liver tissue, samples were fixed and histological analysis was performed as described in [[4]]. Immediately after removal, the electrical conductivity of liver tissue was measured on a Rohde & Schwarz ZNB 40 instrument (Germany). The obtained results were subjected to statistical processing, mean values and standard errors were determined,
differences between control and experimental groups were considered significant at $p < 0.05$.

Results. It was found that at the initial stages of fibrosis development [[6],[7]], there was an increase in the thickness of the Glisson’s capsule [[8]] into which immune-competent cells were incorporated (Fig. 1).

In the intact control tissue sample (Fig. 1a), the hepatic beams retain their structure and consistency (1). The hepatic lobules retain their structure. Partial autolysis of hepatocytes is observed (2) and Dysse space is expanded (3). The central vein is blood-filled. The Glisson’s capsule is thinly expressed (9).

Experimental animals with Cu-induced fibrosis (Fig. 1b) demonstrate partial autolysis of hepatocytes (2), and discomplexation of hepatic beams is frequently seen (1). The vessels are blood-filled (8). Dysse space is reduced (3), in which Ito cells are found (4). Fibroblasts are few, endotheliocytes are found in moderate numbers most often near blood vessels. The endothelium is terminating. The Glisson’s capsule is significantly thickened (9).

Multiple ruptures were observed after treatment of animals with Cu-induced mixed-factor fibrosis (Fig. 1 c). The hepatic lobules are disrupted. The vessels are blood-filled (8). Dysse space is expanded (3), Ito cells are quite frequent (4). Endotheliocytes (5), few lymphocytes (6) and fibroblasts (7) are seen in the surrounding blood vessels. The Glisson’s capsule (9) remains as increased (Fig. 1 c).

Fig. 1. Histological liver preparations, a – intact control, b – animals with Cu-induced fibrosis, c – animals with Cu-induced fibrosis, treated mix factor, d – animals with Cu-induced fibrosis, treated chlorogenic acid.
In animals with Cu-induced fibrosis treated with chlorogenic acid, (Fig. 1 d) there is a pronounced discomplexation of the hepatic beams (1) and complete autolysis of hepatocytes (2). The nuclei are of approximately the same shape and size, rounded. A moderate number of lymphocytes can be observed (6). Dysse space is reduced (3), Ito cells (4) are seen most often in the area of hepatic lobules. Multiple fibroblasts (7) and endotheliocytes (5) are found on the preparation, most often located near vessels. Multiple ruptures are observed over the entire preparation. (Fig. 1 d).

Consequently, at the initial stages of fibrosis development histological changes are pronounced insignificantly, the most pronounced is the thickening of Glisson’s capsule. On the background of chlorogenic acid administration, pronounced autolysis of hepatocytes is observed.

Measurement of the whole tissue electrical conductivity showed that it was slightly increased in liver with Cu-induced fibrosis (Fig. 2 a). When animals with fibrosis were injected with the mix factor, the electrical conductivity remained above the intact control but did not differ from Cu-induced fibrosis (Fig. 2 a). After administration of chlorogenic acid, the electrical conductivity of whole tissue was significantly higher than intact control but did not differ from other variants of hepatic tissue (Fig. 2 a). Consequently, possible changes in the structural and functional organisation of the liver after administration of hepatoprotective substances were not altered compared to fibrosis. At the same time, Cu-induced fibrosis led to structural-functional changes, which influenced the insignificant change of electrical conductivity.

![Fig. 2. Electrical conductivity of: a – liver tissue and, b – homogenised liver of animals of four experimental groups: 1 – intact control, 2 – animals with Cu-induced fibrosis, 3 – animals with Cu-induced fibrosis treated with mix factor, 4 – animals with Cu-induced fibrosis treated with chlorogenic acid. The mean data and standard errors are given. The asterisk indicates variants for which P < 0.05 compared to the control.](image)
The obtained results can be explained by the fact that an increase in the thickness of the liver capsule and, as it was shown in other studies [9], connective tissue, can influence the electrical conductivity. In order to exclude possible influence of the capsule and connective tissue formations, the investigated tissues were exposed to soft homogenisation.

It was found that the electrical conductivity of liver homogenate with fibrosis was significantly higher than that of whole tissue and significantly higher than that of tissue homogenate of intact animals (Fig. 2 b). Administration of mix factor to animals with fibrosis had no effect on the electrical conductivity of tissue homogenate compared to fibrosis (Fig. 2 b). At the same time, administration of chlorogenic acid to the animals with fibrosis resulted in a significant decrease (to the level of the initial control) in the electrical conductivity of the homogenate. Consequently, connective tissue, Glisson's capsule and other structural components influence the electrical conductivity. Structural and functional changes in Cu-induced fibrosis influence electrical conductivity and may be of interest after further studies as one of the indicators of liver function and structure.

References: