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Morphological State of the Liver During Free Radical Processes in Experimental Brain Ischemia

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Abstract. This article is based on the experimental data on the assessment of the mechanism of reperfusion damage to the brain in incomplete ischemia. Vascular disease has become an epidemic and approximately every third person after 40 years has a disease of the vascular network. The authors of the article carried out the experimental part in accordance with the requirements of the International Rules for the Humane Treatment of Animals, as reflected in the Sanitary Rules for the Equipment and Maintenance of Experimental Biological Clinics (Vivariums). The animals used weighing 250-280 grams at the age of 4-7 months were divided into 2 groups and the studies were carried out 1, 3 and 7 days after ischemia-reperfusion.

Key words:hypercholesterolemia; coronary heart disease;ischemia; blood cells; pathology of the cardiovascular system; heart failure; atrial fibrillation; brain; hypoxia.

INTRODUCTION

With cerebral ischemia, hypoxia plays the role of a central link in pathogenesis and, due to severe consequences, poses a threat to the patient's life. The main consequence of hypoxia is energy deficiency, which further determines the development of a pathobiochemical cascade of neuronal damage.

The limitation of oxygen delivery by blood or its delivery from blood to tissue cells due to disruption of the respiratory chain of mitochondria, depletion of cellular reserves of macroergs leads to a slowdown in metabolic processes due to bioenergy disorders [2, 4, 6].

The main risk factors for ischemic stroke are: arterial hypertension, diabetes mellitus, decreased physical activity, smoking, alcohol abuse, pathology of the cardiovascular system (heart failure, coronary heart disease, atrial fibrillation), hypercholesterolemia, as well as homocysteinemia - a likely risk factor that attracts great attention [8, 14]. Metabolic changes in response to the development of acute focal cerebral ischemia develop in a natural sequence. With a decrease in cerebral blood flow below 55 ml / 100 g of tissue in 1 minute, inhibition of protein synthesis in neurons begins, below 35 ml / 100 g anaerobic glycolysis is activated, and a further decrease in blood flow to 20 ml / 100 g of tissue leads to a pronounced violation of energy metabolism, anoxic depolarization of cell membranes with a high probability of cell death. In the area of the brain with a marked decrease in blood flow (less than 10 ml / 100 g per 1 min) within 6-8 minutes from the development of acute ischemia, irreversible damage develops with the formation of a heart attack heart (central zone) ("core", or "nuclear" zone , ischemia), and within a few hours a zone of ischemia is

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formed around the central zone, still viable tissue (with a blood flow above 20 ml / 100 g per 1 min) - the so-called zone of "ischemic partial shade", or penumbra (penumbra) [6, 12].
The concept of "cerebral ischemia" implies a dynamic process and the potential reversibility of the changes caused. For several hours, the central "point" heart attack is surrounded by the zone of "ischemic penumbra", or penumbra, in which energy metabolism is generally preserved and only functional, but not structural changes are present. [5, 9].
According to recent studies, the staging of hemodynamic and metabolic changes occurring in the brain tissue at various stages of insufficiency of its blood supply is shown [3, 11].
Current literature suggests that the presence of liver dysfunction is an independent factor in the increased risk of cerebral ischemic stroke [15, 16].
It is known that the main damaging factors that can inhibit the function of the liver include, first of all, all situations that violate the hepatic blood flow [1, 4, 6]. A damaged liver in itself can change the course of many conditions and the metabolism of the body as a whole, including the pharmacological effect of drugs [3, 2, 8].

Purpose Of The Study:
to assess the morphological status of the liver and some processes of generation of reactive oxygen species (ROS) in whole blood and serum in an experimental model of incomplete rat brain ischemia**.**

MATERIALS AND RESEARCH METHODS:
this article is based on experimental data on the assessment of the mechanism of reperfusion damage to the brain with incomplete ischemia. The experimental part was carried out in accordance with the requirements of the International Rules for the Humane Treatment of Animals, as reflected in the Sanitary Rules for the Equipment and Maintenance of Experimental Biological Clinics (Vivariums).
Used animals weighing 250-280 grams at the age of 4-7 months were divided into 2 groups: the 1st group consisted of 8 rats, which underwent a skin incision of the neck region above the carotid artery on one side (left), followed by suturing of the skin (falsely operated), The second group consisted of 9 rats, which opened the left carotid artery, was clipped for 20 minutes, followed by reperfusion and complete restoration of cerebral blood flow.
Studies were carried out 1, 3 and 7 days after ischemia-reperfusion. Verification of ischemic stroke was confirmed on the basis of viewing light-optical preparations stained by Nissl and hematoxylin-eosin. To obtain semi-thin sections, pieces of tissue in the temporal region of the brain were fixed in 2.5% glutaraldehyde, followed by standard methods in increasing concentrations of alcohols and pouring into araldite. Semi-thin sections were obtained on an LKB ultramikrotome, stained with methylene blue and fuchsin.
The concentration of malondialdehyde was determined by the method of Uchiyama M., Mihara M. [14]. 0.25 ml of blood serum was added to 3 ml of 1.4% phosphoric acid, then 1 ml of a 0.5% solution of thiobarbituric acid was poured and placed in a boiling water bath for 45 minutes. Samples were cooled, 4 ml of butanol was added and shaken for 1 min until a suspension formed. After centrifugation, the supernatant was photographed at two wavelengths $\lambda = 535$ nm and $\lambda = 570$ nm against a blank sample in a cuvette with a long optical path of 1 cm. The content of TAC-active products was calculated using the formula $C = (D_{535} - D_{570}) / 0.156 \times 16$, where C is the

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concentration of TBA-active products in the experimental sample; D_{535} - optical density of the sample at 535 nm; D_{570} - optical density at 570 nm; 0.156 is the molar extinction coefficient of the malonicdialdehyde-TBA complex in l / μmol / cm; 16 - serum dilution ratio.

RESEARCH RESULTS:

during macroscopic examination, we found that the liver has a smooth surface, a rounded front edge, focal hyperemia and some thickening of the capsule are noted. With protracted and severe ischemic brain damage for a long time in older animals, liver compaction with elements of a "septal pattern" on its surface is observed (Fig. 1.).

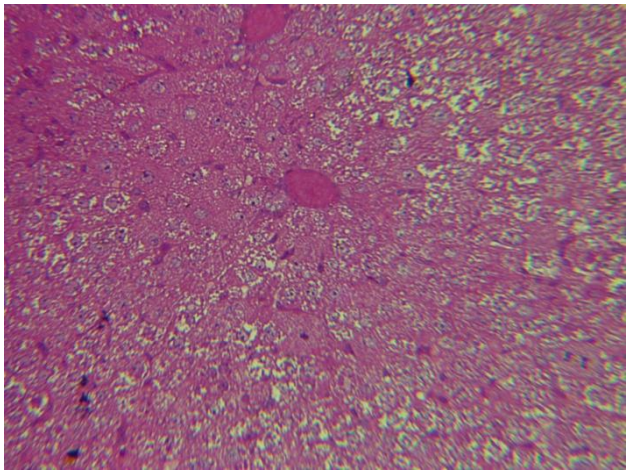

Fig. 1. Rat liver

Nissl and hematoxylin-eosin stain. Electron micrograph, magnification x 400. Intact rat liver in control.

We also observed the presence of small confocal areas of the inflammatory reaction and dystrophic changes, mainly in the portal tracts characterized by elements of small focal periportal hepatitis (Fig. 2.)

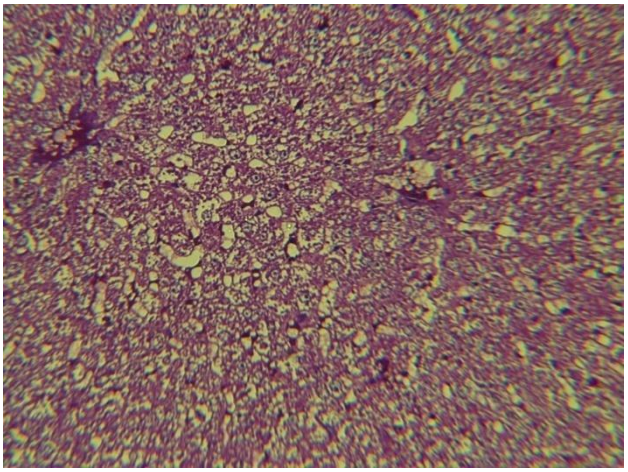

Fig. 2. Rat liver

Nissl and hematoxylin-eosin stain. Electron micrograph, magnification x 400.
Rat liver, 1 hour after unilateral ischemia-reperfusion of the brain.

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In a morphological study in rats with simulated cerebral ischemia, we observed single small-point inflammatory infiltrates, which were rarely located and left the portal stroma in the peripheral sections of the lobule without the development of hepatocyte necrosis (Fig. 3).

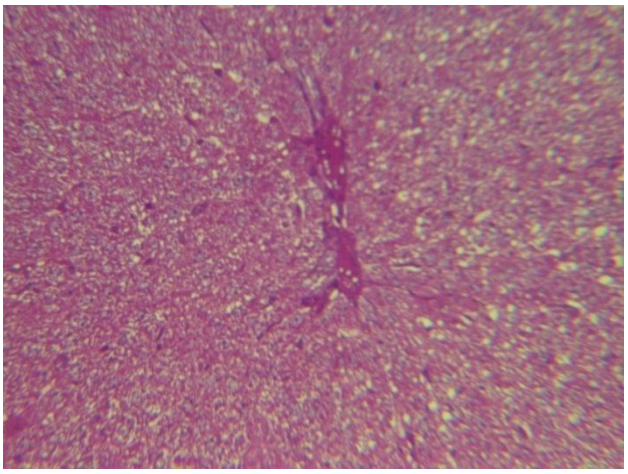

Fig. 3. The liver of the rat

Nissl and hematoxylin-eosin stain.
Electron micrograph, magnification x 400.
Rat liver, 24 hours after unilateral ischemia-reperfusion of the brain.

Small-point inflammatory infiltrates were located between the liver cells - the so-called discrete infiltrate. Also, occasionally developing single periportal necrosis was observed. In the early stages of experimental ischemic stroke in rats, focal proliferative changes within the lobules were observed in the form of clearly delimited infiltrates from derivatives of the mononuclear phagocyte system.

The intensity of the hematotissue metabolism largely depends on the speed of blood flow in the sinusoids (Fig. 5.), which in turn envy is associated with the peculiarities of their structure. So we canceled that normal and first day in the periportal sections of the liver lobule during an experimental ischemic stroke, mainly direct and branching sinusoids are found (Fig. 1-3). Subsequently, by 7 days, the periportal tracts to the adjoining zones to the central veins are characterized by predominantly branching sinusoids with a clear tendency to anastomosis, a characteristic feature of which we have revealed is a directly proportional relationship between the appearance of unulipody and the degree of cerebral ischemia (Fig. 4-5.).

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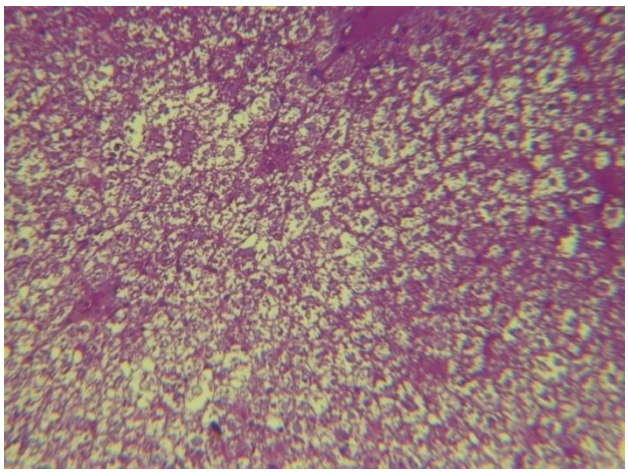

Fig. 4. The liver of a rat

Nissl and hematoxylin-eosin stain.
Electron micrograph, magnification x 400.
Rat liver, the third day after unilateral ischemia-reperfusion of the brain.

In the general description, morphological manifestations in ischemic cerebral catastrophes are manifested by a violation of the beam structure of the liver, intralobular alterative manifestations with the manifestation of necrosis of single hepatocytes with the accumulation of a small number of macrophages, lymphocytes, neutrophils in these areas (Fig. 4.), foci of hepatocyte fatty degeneration and proliferation reticuloendotheliocytes, edema and enlargement of portal tracts with infiltration of their lymphohistiocytic elements and neutrophy sludge, sometimes proliferation of periportal and intralobular bile ducts and the formation of lymphoid follicles (Fig. 5.).

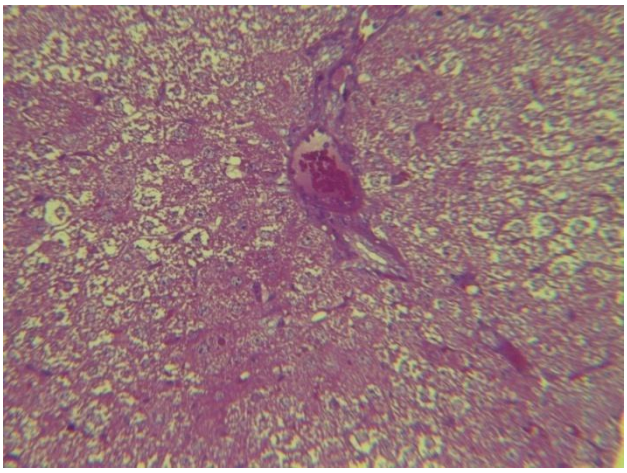

Fig. 5. The liver of the rat
Nissl and hematoxylin-eosin stain. Electron micrograph, magnification x 440.
Rat liver, seventh day after unilateral ischemia-reperfusion of the brain.Severe intravascular stasis, swelling of the connective tissue structures.

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In our studies of experimental ischemic stroke, a tendency toward centralization of intrahepatic blood circulation was noted due to the presence of port-portal anastomoses inside the hepatic lobes and collaterals.

Microscopically revealed polymorphism of hepatocytes (cells of various sizes, among them a large number of binaries and multinucleates, nuclei of various sizes), their swelling, as a result of which the clarity of the beam structure is impaired.

Protein (hydropic, balloon) and fatty degeneration are of small focal nature, and the severity of these changes is difficult to determine as characteristic or specific in a particular case.

In various sections of the hepatic lobules, small foci of parenchyma necrosis are found with destruction of the argyrophilicstroma and focal infiltrates from macrophages, lymphocytes, and neutrophils. Proliferation and hypertrophy of stellate reticuloendotheliocytes (liver macrophages) are expressed. The portal tracts are enlarged, edematous, moderately or slightly infiltrated by lymphohistiocytic elements with an admixture of neutrophils.

Circulatory disorders that occur with ischemic brain damage cause increased generation of reactive oxygen species (ROS), and, as a result, increased LPO. Data on the study of the content of thiobarbituric acid reactants (TBA-AP) are presented in Table. 1.

Table 1.

The dynamics of the concentration of TBA-AP (MDA) in serum when modeling brain ischemia (nmol / mg protein * min)

Effect on the brain	**Time after ischemia**			**Control**
	2 hour	**1 day**	**3 day**	
Occlusion	1,99* (1,23-2,76)	3,0* (2,43-3,58)	2,6* (1,84-2,7)	1,12 (1,1-1,2)
Reperfusion	3,9* (2,46-5,4)	3,1* (2,46-3,7)	3,1* (2,46-3,48)	

* - significantly compared to control , p <0,05

When studying the dynamics of TBA-active products, a significant increase (in comparison with the control group of animals) of MDA concentration in all series of the experiment was revealed. A significant increase in the concentration of MDA after occlusion was noted at all stages of observation, however, the greatest changes (2-5-3 times) were detected after 24 and 72 hours. Reperfusion processes were accompanied by a significant increase (3 times) in the concentration of MDA 2 ,24 and 72 hours after reperfusion.

Thus, the study of lipid peroxidation indices in modeling ischemia revealed the activation of this process. Reperfusion processes in the brain were accompanied by the most significant changes from the studied parameter.

DISCUSSION.

Cerebrovascular disorders occupy one of the leading places in world statistics and Uzbekistan in morbidity, disability and mortality. On average, about 400-450 thousand cerebral strokes

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are registered annually in Uzbekistan (80-85% of ischemic strokes), of which up to 200 thousand are fatal [1, 5, 7]. Among stroke survivors, at least 75% have persistent disabilities. In recent years, there has been a tendency to increase the frequency of stroke in people of working age. A stroke, as a rule, develops against the background of chronic brain ischemia, i.e. is a specific stage of cerebrovascular disease [13,18].

Many authors have repeatedly emphasized that in the pathogenesis of an early period of cerebral ischemic stroke, an important role is played by impaired liver function [10, 17], often leading to the death of the patient with progression of positive dynamics of neurological status.

Cerebral ischemia is accompanied not only by hypoxia of brain tissue, but also by increased generation of reactive oxygen species that oxidize macromolecules of lipid and protein nature and the formation of low molecular weight products of their breakdown. The leaching of low molecular weight products into peripheral blood leads to toxic damage to internal organs such as the liver and kidneys, which is accompanied by increased functioning of the detoxification system of these organs. Cerebral ischemia is accompanied by a redistribution of oxygen in the body in favor of the brain, which leads to a decrease in the partial pressure of oxygen in the internal organs, in particular in the liver. Studies on changes in the antioxidant system of the membrane components of the liver in experimental ischemic stroke are very limited.

The macroscopic changes we observe, such as compaction of the liver with a rounded front edge, focal hyperemia and some thickening of the capsule with a smoothed surface, the appearance of “septal pattern” elements on its surface indicate a high likelihood of subsequent development of focal fibrosis.

As a result of our studies, the opinion of many authoritative scientists has been confirmed that in the acute period of ischemic stroke in the liver, the capillaries are narrowed with a slowdown in blood flow and red blood cell aggregation in them, which is of great importance in the mechanism of hepatic circulation disorders. There is also a narrowing of the small veins, a gradual expansion of sinusoids with a slowdown in blood flow and aggregation of red blood cells in them, intrahepatic bypass bypass.

The presence of small confocal areas of the inflammatory reaction with elements of dystrophic changes mainly in the portal tracts and periportal zones, that is, in the 1st zones. It is known that in these zones, in comparison with others, there is the highest content of oxygen and substances for metabolic reactions, and the metabolic and regenerative activity of this zone is the highest, therefore, we believe that these sites are the first to be included in the pathological processes associated with the root cause [11, 17,18].

The expansion of the portal tracts, their swelling and infiltration with lymphohistiocytic structures indicates a further possibility of the development of sclerotic processes in these areas of various, often moderate, severity. Clearly delimited infiltrates from cells characterizing focal proliferative changes inside the lobules in the early stages of experimental ischemic stroke in rats are a manifestation of small-pointed infiltrative granulomatous inflammatory process.

As a result of our studies, it was confirmed that cerebral ischemic catastrophes disrupt the morphological landscape of the liver structures, which is determined not only by the impossibility of fully ensuring “central” control of damaged brain ischemia, but also

manifests itself. Thus, with ischemia of the brain, not only the direct, but also the inverse relationship of the brain-liver system is disturbed.

MDA is known to be a universal marker of LP. According to the literature [4,19], as early as 5 minutes of ischemia, an increase in all lipid peroxidation products — primary and secondary — is recorded. The fact of a sharp activation of ROS generation in the focus of ischemia is confirmed by an increase in MDA.

Activation of free radical processes was noted in all series of the experiment with occlusion. The increase in MDA in rat serum after occlusion indicates an increase in LPO processes in the first hours of the development of the disease. The maximum increase in the concentration of TBA-active products was recorded 1 day after occlusion. In animals, on the 3rd day after 2-hour occlusion, the concentration of MDA also exceeded the values of the control group.

A study of LPO indicators during reperfusion showed that changes were detected at all stages of the experiment, which is consistent with published data on the enhancement of SRO in this period.

CONCLUSION:

Dysfunction of the liver in ischemic disorders of the central regulatory mechanisms is most often diverse, but morphologically not so pronounced. Central disorders of liver function often serve not only as a backdrop on which more severe diseases of this organ develop later on under the influence of infection, intoxication, and other errors, and often can play the role of an activator of aggravation of the severity of the condition as a whole.

Occlusive and reperfusion processes in modeling cerebral ischemia were accompanied by an increase in free radical processes. The concentration of MDA in rat serum increased significantly in the first day after occlusion, as well as in all observation periods after reperfusion.

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