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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

MORPHOLOGICAL AND BIOCHEMICAL CHANGES IN THE LIVER ASSOCIATED WITH REPERFUSION SYNDROME, THE ROLE OF ANTIOXIDANT DEFENSE SYSTEM IN THEIR PATHOGENESIS

Specialty:	3242.01 – "Pathological anatomy"
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Applicant:	**Mirhafiz Ibrahim Mirzayev**

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The dissertation work was performed at the Scientific Research Center of Azerbaijan Medical University and the Department of Pathological Anatomy.

Scientific supervisor: Doctor of Medical Sciences, Professor **Adalat Reyhala Hasanov**

Official opponents:

Mehmet Akif Chiftchioglu

Philosophy doctor in medicine, dosent
Fikrat Xanhuseyn Aliyev

Philosophy doctor in medicine
Shahin Shalbuz Osmanov

Dissertation Council BED 4.20 of the Supreme Attestation Commission under the President of the Republic of Azerbaijan, operating at the Azerbaijan Medical University

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GENERAL DESCRIPTION OF THE STUDY

The relevance of the study. Liver diseases, including cirrhosis of the liver, widespread liver abscesses, cancer and other diseases today are at the forefront among diseases of the abdominal cavity organs and often result in death. Therefore, the development of more effective methods of treatment of liver diseases has become one of the priority areas of medical science.

By bringing this problem to the forefront, liver cancer, liver cirrhosis and ischemia, which are considered to be beyond timely treatment, have already been solved by the application of liver transplantation in practical medicine[1]. Today, liver transplantation is considered a radical treatment for cirrhosis and liver cancer. However, despite all these positive results, liver transplantation and the occurrence of a number of problems in subsequent periods create a number of problems in the occurrence of the therapeutic effect of this progressive method. One of such problems is reperfusion syndrome after transplantation[2]. It is known that during liver transplantation, as well as during its extensive resection, the vessels feeding and exiting from this area are temporarily clamped to prevent acute bleeding. At this time ischemia occurs in the organs whose nutrition is temporarily stopped, and in the bloodless region ATP, considered the main source of energy in hepatocytes, Kupffer and endothelial cells, is rapidly consumed. As a result, toxic metabolites appear and accumulate in the intercellular space[3].

After surgergical operation, starting from the moment of restoration of blood flow, the toxic products formed there circulate in the body and create endogenous intoxication, disrupting the balance

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[1] Андрейцева, О.И. Трансплантация печени при первычном раке. // – Тез. Док-ов Здоровье столицы 2008. – с.203-204.

[2] Ходосовский, М.Н. Коррекция окислительных повреждений при синдроме ишемии-реперфузии печени. // – Журнал Гродненского гос. Медицинского университета 2016, № 4, – с.20-25.

[3] Contaldo, C., Elsherbiny, A., Lindenblatt, N. [et al.] Erythropoietin enhances oxygenation in critically perfused tissue through modulation of nitric oxide synthase. //– Shock, 2009, Vol.31, № 6, – p.599-606.

of a number of organs, including hepatic metabolism[4]. In cases where the amount of toxic substances formed as a result of metabolic disorders reaches an extreme level, intoxication turns into toxemia, leading to toxic shock and even organ failure[5]. All these factors worsen the results of reconstructive-recovery surgery, and in some cases make it useless. In this regard, numerous scientific studies on the pathogenesis and prevention of reperfusion syndrome have been conducted. As a result of numerous studies, it has been established that liver damage due to reperfusion proceeds in 2 phases. Phase 1 is early and includes a 6-hour period of restoration of impaired blood circulation. During this period, hepatocytes, Kupffer cells, and sinusoidal endothelial cells are damaged to varying degrees due to the effects of toxic substances ingested during reperfusion. After 6 hours, cytokines and chemokines are activated in the liver tissue as a result of reperfusion, followed by inflammation in the liver. Due to the development of the inflammatory process, ion exchange in the liver tissue is disturbed, the functional state of the microcirculatory network is weakened, and other complications develop.

Thanks to the rapid introduction of new technologies into medical practice, liver diseases that were not amenable to timely treatment were resolved through liver transplantation. However, a number of problems encountered during liver transplantation, especially post-transplant reperfusion syndrome, prevent effective results from this advanced treatment method[6].

It has already been unequivocally proved that metabolites formed in the ischemic focus of the liver during liver transplantation surgery spread throughout the organs and tissues when blood flow is restored and disturb the metabolism in the body, and the resulting imbalance creates

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[4]Гринев, М.В., Гринев, К.М. Цитокин - ассоциированные нарушения микроциркуляции (ишемический – реперфузионный синдром) в генезе критических состояний. //– Хирургия, им. Н.И.Пирогова, 2010, №12, – с.70-76.

[5]Алиханов, Р.Б., Кубышкин, В.А. Патофизиологические аспекты реперфузионных повреждений печени. //– Кубанский научный медицинский вестник 2013. № 7, – с.170-173.

[6] Ходосовский0 М.Н. Коррекция окислительных повреждений при синдроме ишемии-реперфузии печени. // Журнал Гродненского Гос. Медицинского Университета 2016 №4. Стр. 20-25.

certain obstacles to the operation of the transplanted liver[7]. In spite of numerous studies to solve these problems, the complex connections between structural changes and biochemical and biophysical processes occurring in organs during reperfusion have not been elucidated.

The main objective of the research work: to study the relationship between the intensity of oxidative stress in liver tissue and changes in hepatocyte structure depending on the duration of reperfusion syndrome and, based on this, to prepare appropriate preventive measures.

Objectives of the study:

1. Determination of oxidative stress in liver tissue depending on the duration of ischemia;
2. Determination of the state of the antioxidant defense system in hepatocytes depending on the duration of ischemia;
3. Study of changes in hepatocytes, depending on the state of oxidative stress in the liver;
4. Study of changes in the state of oxidative stress in liver tissue and the structure of hepatocytes, depending on the duration of reperfusion;
5. Study of the effect of increasing the overall antioxidant defense system of the organism on the changes that reperfusion creates in liver tissue.

Research methods. Models of ischemia and reperfusion were created in the liver of white rats. After completion of the experiments, homogenate was prepared from the liver. The process of lipid free-radicalization and the state of antioxidant defense system were studied in the homogenate. In addition, histological preparations were prepared and microscopic examination was performed by taking liver pieces from liver sections subjected to ischemia and reperfusion. The obtained quantitative indices were processed statistically using Student's t-criterion based on current recommendations, as well as nonparametric Wilcoxon-Mann-Whitney U-criterion. Correlation analysis of the interaction between the studied indicators was performed using the Breiv-Pearson method.

[7] Eipel C. Regulation of hepatic blood flow: the hepatic arterial buffer response revisited. Eipel C. Abshagen K. and Vollmar B. // World journal of qastroenterology. 2010. Vol 6 №48 p.6046-6057.

Main provisions submitted for defense:

1. The intensity of oxidative stress in the liver increases in parallel with the duration of ischemia, and as the duration of ischemia increases, the violation of the membrane of hepatocytes around the central vein forms the basis for the development of the ischemic process.
2. During compression of the hepatic artery, the antioxidant defense system in the liver tissue is directed towards weakening, but it does not take a serious character for 15 minutes, but it includes all animals that are experimented with, as well as deepening in 30 minutes.
3. The intensity of oxidative stress generated in liver tissue during reperfusion is correlated with the duration of reperfusion and ischemia.
4. Strengthening the antioxidant defense system of the organism in the early stages of ischemia significantly reduces the destructive effect of the developed reperfusion syndrome on hepatocytes during the restoration of blood flow.

The scientific novelty of research work. The role of oxidative stress in liver tissue in the mechanism of reperfusion syndrome development has been revealed. The role of surface and intrastructural sulfhydryl groups of proteins, which are the main links of antioxidant defense system, in the process of hepatocyte membrane damage in reperfusion syndrome has been proved. The optimal duration of ischemia for treatment of complications of reperfusion syndrome was determined.

Theoretical and practical significance of the study. Theoretical significance of the study is determined by the fact that the obtained results will further clarify and expand the available information on reperfusion syndrome and its complications in liver transplantation. The obtained information can be used in the educational process of the departments of pathologic physiology, pathologic anatomy, biological chemistry and surgical diseases of AMU. Theoretical bases of using the strengthening of antioxidant defense system of the organism in the optimal interval of ischemia as the main means of prevention of destructive effect of reperfusion syndrome on hepatocytes have been developed. The role of surface and intrastructural protein sulfhydryl group in hepatocyte membrane disintegration in reperfusion syndrome has been revealed, and the prospective use of new selective drugs has been proved.

Aprobation of the research work. Separate fragments of the research work were discussed in the following scientific meetings:

Scientific-practical conference dedicated to the 92nd anniversary of National Leader Heydar Aliyev (Baku 2015).

Turaz Academy, Turkey-Azerbaijan 1st International Forensic Medical expertise and pathology congress. October 13-16 (Baku 2016).

Science and Education Materials of the XI International research and Practice conference. April 6th-7ti Munich 2016 Germany.

Materials of the Scientific-practical conference dedicated to the 25th anniversary of restoration of Azerbaijan's state Independence (Baku 2017).

Scientific-practical conference dedicated to the 100th anniversary of the Azerbaijan Democratic Republic (Baku 2018).

International Scientific-practical conference dedicated to the 100th anniversary of the Department of Human Anatomy and Medical Terminology of Azerbaijan Medical University December (Baku 2019).

Joint Scientific Meeting of the Scientific Research Center, the Association of Pathological anatomy, the Department of Pathological anatomy (Baku 2021).

At the scientific seminar of the Dissertation Council BED 4.20 at Azerbaijan Medical University (Baku 2024).

The results of the study were recommended for use in the educational process and relevant lectures at the Departments of Pathological Anatomy, Pathological Physiology, Biochemistry and Surgical diseases of Azerbaijan Medical University.

Published scientific works. On the subject of dissertation work 14 scientific works have been published. From them 7 scientific articles, 7 conference materials. 1 article and 1 conference material were published in foreign press ("Bulletin of Surgery of Kazakhstan") and ("Scientific and Educational Materials of XI International Scientific and Practical Conference" Munich, Germany). 6 articles were published in journals recommended by the Supreme Attestation Commission under the President of the Republic of Azerbaijan. 2 of them (Republic) were published in a periodical scientific edition included in the international system of summarization and indexing (SCOPUS) (Azerbaijan Medical Journal 2017 №3, 2018 №4). 1 article (Bulletin of Surgery of

Kazakhstan, 2019) was published abroad, in a scientific edition included in SCOPUS. Two articles and one thesis are monoauthored.

Volume and structure of the dissertation. The dissertation work consists of 185 pages (219055 characters) typed on a computer and consists of an "Introduction" (volume: 13620 characters), "Main content of the dissertation" (volume: 26757 characters), "Conclusion" (volume: 15485 characters), "Results" (volume: 2841 characters), "Practical recommendations" (volume: 909 characters), "List of used literature" and "Abbreviations".

The "Main content of the thesis" is divided into 6 chapters: Chapter I. "Literature review" (volume: 26757 characters), Chapter II. "Materials and methods of research" (volume: 12562 marks), Chapter III. "Changes in liver tissue depending on the duration of ischemia" (volume: 22098 marks), Chapter IV. "Changes in liver tissue due to reperfusion after different periods of ischemia" (volume: 51531 marks), Chapter V. "Ischemia-induced changes in liver tissue under conditions of enhanced antioxidant defense system" (volume: 18633 characters), Chapter VI. "Results of reperfusion in experimental animals with enhanced antioxidant defense system" (volume: 57368 characters).

The list of used literature includes 196 sources, 13 of them in Azerbaijani, 94 in Russian and 89 in other languages. The research work is illustrated with 41 figures, 15 tables and 3 diagrams.

MATERİALS AND METHODS OF RESEACH

The research work was carried out in the Scientific Research Center and Pathological Anatomy Department of Azerbaijan Medical University.

Experiments were conducted on 160 male white rats weighing 200-250 grams in strict compliance with the rules of the Helsinki Declaration on the treatment of experimental vertebrate animals of the European Committee on Bioethics, adopted in Strasbourg in 1986.

The experimental animals were divided into 8 groups (Table 1). After completion of the experiments, each of them was injected with 1 ml of calypsol solution, the liver was removed under complete anesthesia, and the intensity of free lipid peroxidation (FLP) and markers of the

antioxidant defense system (ADS) were determined in the prepared homogenate. The concentration of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) from FLP markers was determined by the method of Asakawa T, Matsishita S[8]. diene conjugates (DC) - by the method developed by I.D. Stalnaya[9].

Table1.

Grouping of experimental animals

№	Groups	Experiments	Number
1.	I	Intact condition	5
2.	II	Creation of a liver ischemia model	15
3.	III	Reperfusion after 5 minutes of ischemia	25
4.	IV	Reperfusion after 15 minutes of ischemia	25
5.	V	Reperfusion after 30 minutes of ischemia	25
6.	VI	An ischemia model was created 4 hours after intramuscular injection of mexidol	15
7.	VII	Reperfusion was performed in the 5th minute of ischemia created after intramuscular injection of mexidol	25
8.	VIII	Reperfusion was performed in the 30th minute of ischemia created after intramuscular injection of mexidol	25

Among the markers of ADS, surface and structural proteins of SH (sulfohydryl) groups were determined by Ellman method[10], total antioxidant activity (TAA) by Benzi and Strain method[11] reduced concentration of glutathione and catalase by the Bergmeyer method[12].

[8] Asakawa T., Colorig condition of TBA test for detecting lipid hydroperoxides Asakawa T., Matsushita S. // Lipids 1980, №3, p 137-140

[9] Стальная И.Д. Метод определения диеновой конюгации ненасыщенных выших жирных кислот. // Современные методы в биохимии М. Медицина 1997, стр.63-64.

[10] Ellman G. Tissue sulfhydryle qroups // Archives of biochemisty and Biochysics, 1959, vol 16, №48. p.6046-6057.

[11] Benzie I.F. The ferric reducing ability of plasma (FRAP) as measure of antioxidant power the FRAP assay Benzie I.F., Strain I.I. // Anal.Biochem 1996 №1.p.70-76.

[12] Benzie I.F. The ferric reducing ability of plasma (FRAP) as measure of antioxidant power the FRAP assay Benzie I.F., Strain I.I. // Anal.Biochem 1996 №1.p.70-76.

Changes in the structure of the liver were studied by microscopic examination of preparations stained with hemotoxylin and eosin, carmine stain (Best's method).

The obtained quantitative indicators were statistically processed using Student's t-criterion based on modern recommendations, as well as nonparametric Wilcoxon-Mann-White U-criterion. Correlation analysis of the interaction of the studied indicators was carried out using the Breiv-Pearson method. The obtained results are recorded in a spreadsheet.

RESEARCH RESULTS AND THEIR DISCUSSION

It was found that the H_2O_2 concentration in the liver of intact white rats (group 1) was 2,46±0,14 c.u., DC concentration was 1,418±0,11 v/ml, and the concentration of MDA was 1,55±0,02 nmol/mg.

The activity of surface SH-groups of proteins is 33,5±0,7 nmol/mg, the concentration of intrastructural SH-groups of proteins is 22,7±0,7 nmol/mg, the concentration of reduced glutathione is 13,2±0,4 nmol/mg, the concentration of catalase is 265,9±0,57 c.u., and the activity of TAA is 40,6±0,3%.

The structure of the liver of intact white rats was in normal condition; hepatocytes formed anastomoses with each other and were arranged radially from the central vein in the form of hepatic beams. Each of the hepatic beams consisted of two transverse hepatocytes. Between the hepatocytes forming the beams are bile ducts, and between them are sinusoids. Blood passes through the sinusoids and travels toward the *v. centralis* between the liver's beams. The wall of the sinusoids is composed of sinusoidal cells (endotheliocytes, stellate reticuloendotheliocytes, and lipocytes). Sinusoids do not have a basement membrane. In intact animals, it is difficult to visualize because the Disse space is lined with reticular fibers. Nevertheless, reticular fibers form the base of liver lobules.

Microscopy of the liver tissue of intact animals shows that the liver lobes are conventionally divided into the central, intermediate, and periportal parts, located around *v.centralis*.

Portal tracts are formed by the terminal branches of afferent blood vessels (v. porta, a. hepatica), surrounded by connective tissue

fibers and biliary tracts, draining bile from the lobules. The portal tract is also surrounded by lymphatic vessels and nerve fibers.

In experimental animals included in Group II, 5 minutes after ligation of the hepatic artery, H_2O_2 concentration in the liver homogenate increased by 22% ($p<0,01$), MDA concentration by 6,4% ($p<0,01$), and DC concentration by 4,7% ($p<0,01$). This shows that despite the short duration of ischemia, FLP is directed towards intensification. Accordingly, the ADS tends to be attenuated. However, at the 5th minute of ischemia, the decrease in the concentration of ADS markers in the liver tissue was not as noticeable (Table 2).

Experimental animals included in Group II were subjected to ischemia for 5 minutes, while light microscopic examination of these experimental animals did not reveal significant structural changes in the liver tissue or cells composing the tissue. Thus, hepatocytes formed anastomoses with each other and were arranged radially to the central vein in the form of hepatic beams, and no damage to the histoarchitectonics of the liver was observed (Table 2).

Table 2.

Dynamics of change the concentration of FLP and ADS markers in the homogenate depending on the duration of ischemia in the liver

№	Markers	İntact state	Duration of ischemia		
			5 min	15 min	30 min
1	H_2O_2	2,46±0,14	3,00±0,07*	3,36±0,09*	4,04±0,14*
2	MDA	1,55±0,02	1,65±0,02 *	1,80±0,03 *	2,33±0,13 *
3	DC	1,418±0,011	1,484±0,013 *	1,668±0,033 *	1,70±0,2 *
4	Surface p.SH	33,5±0,7	33,3±0,7 **	31,4±0,6 ***	29,5±0,8 *
5	Structural protein SH	22,7±0,7	22,4±0,6 **	20,5±0,8 **	18,7±1,1 ***
6	Glutathione	13,2±0,4	13,1±0,4 **	11,7±0,8 **	10,4±0,9 ***
7	Catalase	265,9±0,5	265,4±0,5 **	263,4±0,9 ***	261,6±1,5 ***
8	TAA	40,6±0,3	40,0±0,5 **	38,9±0,6 ***	37,0±0,7 *

Note: * $p<0,001$; * $p< 0,01$; * * $p>0,05$; *** $p<0,05$;

However, starting from the 15th minute of ischemia, the following changes of FLP markers in liver tissue were observed. H_2O_2 concentration increased by 36,5%, MDA by 16,1%, and DC by 18,4% compared to the intact group. A decrease in ADS markers was observed. Thus, surface SH-group protein concentration decreased by 6%, intrastructural SH-group concentration – by 9,6%, reduced glutathione concentration – by 11,5%, TAA concentration – by 4,4%.

However, unlike the indicated markers, the total concentration of catalase decrised by 1%. In contrast to the 5th minute of ischemia, gaps formed at the 15th minute as the connections between hepatic bundle hepatocytes were weakened.

In the 30th minute of ischemia, the concentration of ADS markers continued to increase. The concentracion of surface SH group decreased by 12%, intrastructural SH group by 18%, reduced glutathione density by 21%, TAA concentration by 9% and catalase density by 1,6%. Among FLP markers, H_2O_2 concentration increased by 64% ($p<0,001$), MDA by 50% ($p<0,001$), and DC by 20% ($p<0,001$) compared to the intact group. At 30 minutes, hydropic dystrophy and even fragmentation of the nucleus appeared in most hepatocytes around the central vein. In some hepatocytes, the integrity of the plasmolemma is compromised.

3-cü qrupa daxil olan təcrübə heyvanlarında 5 dəqiqə davam edən işemiyadan sonra reperfuziya aparılmış və 24 saat müddətində qaraciyər toxumasında oksidativ stresin, AMS-in və hepatositlərin strukturunda baş vermiş dəyişikliklər öyrənilmişdir.

Experimental animals included in Group III were reperfused 5 minutes after ischemia and changes in oxidative stress, ADS, and hepatocyte pattern in liver tissue were studied for 24 hours.

It was found that reperfusion after 5 minutes of ischemia increases oxidative stress in the liver in the first 30 minutes. But in later periods, the intensity of oxidative stress in the liver tissue slightly decreases. Thus, at the 30th minute of reperfusion, the concentration of H_2O_2 in liver tissue was increased by 54,5% ($p<0,001$), MDA concentration by 51% ($p<0,001$), and DC concentration by 9% ($p<0,001$) in liver tissue compared to the intact state. 24 hours after reperfusion, this increase was 39% ($p<0,01$), 35% ($p<0,05$), and 12%

(p<0,001), respectively. Changes in ADS markers were also observed. At the 30th minute of reperfusion, the concentration of surface SH-group protein decreased by 1,5%, intrastructural SH-group by 5%, reduced glutathione concentration by 8,3%, and TAA concentration by 5%. However, in contrast to the mentioned markers, catalase concentration decreased by only 1%. At the 24th hour of reperfusion, the decreases were 6,2%, 10%, 8,3%, 7%, and 1% respectively.

After 5 minutes of ischemia, the following changes were detected in the liver tissue, depending on the duration of reperfusion: 15 minutes after reperfusion, the sinusoids and cavities around the portal tract, which constitute the liver and are part of its histologic structure, are filled with blood. Distinctly visible edema of sinusoidal areas, hyaline and hydropic dystrophy of hepatocytes are noticed. The areas around the portal tract are infiltrated with neutrophilic leukocytes. The central vein is filled with blood. After 30 minutes of reperfusion, the marked structural changes somewhat deepened and attracted attention with more clearly expressed signs of ischemia. Thus, the regenerative activity of hepatocytes around the portal tract of the liver was increased, and their nuclei were enlarged. In some hepatocytes, there were signs of karyopicnosis. But, despite this, cell membranes have preserved their integrity. In some areas, liver cells underwent apoptosis. At microscopy of micropreparations prepared from liver tissue 1 hour after reperfusion, a continued plethora of vessels, increased regenerative activity of hepatocytes, and an increased number of large and binucleate hepatocytes were noted, showing distinctive features from the previous days of the study. Sinusoids are plethoric. 3 hours after reperfusion, a greater increase in vascular engorgement was observed in the micropreparations prepared from liver tissue. One of the signs that attract attention during microscopy of preparations is the continuation of regeneration in the cytoplasm and nucleus of hepatocytes. In parallel, the presence of edema was manifested by leukocytic infiltration around the portal tract. Microscopic examination revealed that 24 hours after reperfusion, recovery processes began in hepatocytes, and simultaneously, a decrease in edema in sinusoidal spaces was noted. In some hepatocytes, the recovery process was completely completed. The nuclei located in the center of hepatocytes are hyperchromic; in some places, the number of binucleate hepatocytes is increased. Thus, the

increase in the number of binucleate hepatocytes indicates a more intensive recovery of the functional activity of the liver as well as the tissue towards its normal structure.

Reperfusion after 15-minute ischemia increases the intensity of FLP in liver tissue compared to 5-minute ischemia. Compared to the intact state, the concentration of H_2O_2 in liver tissue at 30 minutes of reperfusion increased by 62% ($p<0,001$), the concentration of MDA by 51% ($p<0,001$), and the concentration of DC by 26%. At the 24th hour of reperfusion, the concentration of LSP products – H_2O_2 in the liver tissue was 63% ($p<0,01$), the concentration of MDA was 89% ($p<0,001$), and the concentration of DC was 34% ($p<0,001$). As can be seen from here, as the duration of ischemia increases, LSP products accumulate more in the liver, and the increase is also observed at the 24th hour of reperfusion.

The results of the effect of reperfusion on liver structure after 15 minutes of ischemia were as follows:

15 minutes after reperfusion, examination of the microscopic sections taken from the liver tissue of the experimental animals included in this group showed slightly enlarged sinusoids of the lobes and a weak plethora of central veins, which differed from 5-minute ischemia. Vacuolar dystrophy was noted in the cytoplasm of hepatocytes, and leukocytic infiltration was noted around the portal tract. The permeability of hepatocyte membranes was slightly increased.

30 minutes after reperfusion, the sinusoids of the lobules were dilated, and the central veins were less plethoric. The presence of granular inclusions in the cytoplasm of hepatocytes located around the central vein, interstitial edema around the portal tract, and hydropic dystrophy in some hepatocytes in the peripheral part of the vein were detected. Glycogen grains were unevenly distributed in the cytoplasm of hepatocytes. 1 hour after reperfusion, microscopy of preparations prepared from liver tissue revealed that the sinusoids of liver cells continue to dilate. The central vein is different from the previous observation days, with a more pronounced plethora. At the 3rd hour of reperfusion, the liver sinusoids are dilated and blood-filled. At the same time, the central vein has dilated and slightly increased its area. In hepatocytes located around the central vein, hydropic dystrophy and shrinkage of hepatocyte nuclei (karyopicnosis) are noted. Also, leukocytic infiltration is visualized

around the portal tract as well as in the periportal area. 24 hours after reperfusion, examination of preparations made from liver tissue after staining with hematoxylin and eosin showed the following picture: The liver tissue acquired the characteristics of a healthy tissue structure, and its cells returned to their normal structure. Along with damaged hepatocytes, the recovery process of most hepatocytes and the reduction of portal tract edema were observed. The sinusoids and central vein were distinguished from other vessels by their plethora.

After reperfusion, there was a corresponding change in the state of ADS in the liver tissue.

At the 30th minute of reperfusion after 5-minute ischemia, compared to the intact state, the concentration of surface SH-group protein in liver tissue decreased by 1,4%, the concentration of intrastructural SH-group protein by 6,8%, the concentration of reduced glutathione by 4,9%, the concentration of catalase by 1%, and the concentration of TAA decreased by 5,2%. As can be seen from here, despite the restoration of blood flow, ADS in the liver tissue could not be compensated, and the concentration of its markers continued to decrease.

In the subsequent terms of examination, the weakening of markers of the antioxidant defense system continued. Thus, at the 24th hour of reperfusion, the concentration of surface SH-group protein decreased by 6,2%, the concentration of intrastructural SH-group protein by 10,1%, the concentration of reduced glutathione by 8,3%, and the concentration of TAA by 7%. However, there were no significant changes in catalase concentration.

Thus, the results of our experiments show that 5-minute ischemia suppresses ADS in liver tissue, and this process continues even after reperfusion. As the duration of ischemia increased, the changes in ADS after reperfusion on this background became more pronounced. Thus, at the 30th minute of ischemia and 15 minutes after blood flow restoration, the concentration of surface SH-group protein was 20% in comparison with the intact state, the concentration of intrastructural SH-group protein was 32%, the concentration of reduced glutathione was 37%, the concentration of catalase was 3,7%, and the concentration of TAA decreased by 16,8%. At later stages of reperfusion (30th and 60th minutes), the concentration of ADS markers continued to decrease.

Although this process continued at the 3rd hour of reperfusion, it was somewhat milder than in the previous observation periods. At the 24th hour, although it began to compensate, it was still below the level of intact experimental animals. The results of our experiments have shown that restoration of blood flow immediately after ischemia attenuates ADS in liver tissue, and this process becomes more pronounced as the duration of ischemia increases.

After 30 minutes of ischemia and 15 minutes after restoration of blood flow, microscopy of preparations prepared from the liver showed that the vessels forming the portal tract and central vein were filled with blood. The periportal and perivascular septa were mildly edematous, and mucoid swelling was observed. On microscopic examination, although the liver tissue retained lobular structure, mild disorganization of the septa was noted. The sinusoids are enlarged and mosaic. Edema in the Disse space is pronounced. Dystrophic and necrobiotic changes in hepatocytes were more profound than in samples taken from other groups of animals included in the study. The cytoplasm of some hepatocytes located in the portal area were homogenized, and their nuclei were stained hyperchromic. However, the karyolemma of the nuclei did not disintegrate. More severe changes occurred in hepatocytes around the central vein than in hepatocytes in the portal tract. Thus, hydropic dystrophy and nuclear fragmentation are detected in hepatocytes. In one group of hepatocytes, the integrity of the plasmalemma was disturbed. During the microscopic examination of preparations made from liver tissue taken 30 minutes after reperfusion, the continuation of dystrophic and necrobiotic changes in hepatocytes was clearly seen. Thus, the sinusoids and the central vein are enlarged, attracting attention with their plethora. Edema and plethora increased in the portal tract as well as in the periportal area. The number of binucleated hepatocytes also increased. 1 hour after reperfusion, microscopic examination of the hepatocytes shows continued hyaline-droplet and hydropic dystrophy. Fullness and dilatation of the sinusoids were also repeated in this subgroup. At the same time, in the portal tracts, the dilatation of the vessels and the development of interstitial edema around them are observed. The number of binucleated hepatocytes increased slightly.

3 hours after reperfusion, microscopic examination of the liver preparations reveals dilated sinusoids. The plethora of sinusoids and central veins and their dilation resulted in a change in the mutual position of the periportal area. In addition, Lymphocytic infiltration is clearly visible around the portal tract, and multinucleated hepatocytes are prominent. Twenty-four hours after reperfusion, a decrease in interstitial edema around the portal tract, partial recovery of hepatocytes and their cellular components, and increased regenerative activity were found. The integrity of reticular fibers was preserved, and venous congestion of the sinusoids and central veins was noted. The "dark cytoplasmic signs phenomenon," reflecting the acceleration of protein synthesis in hepatocytes, can be considered pathohistological evidence confirming this. However, glycogen dispersed in the cytoplasm of hepatocytes was unevenly distributed, and its amount decreased compared with previous observations. The amount of glycogen also decreased with increasing ischemia time.

The intensity of oxidative stress in the liver tissue was markedly reduced starting from the 5th minute of ischemia in white rats with an enhanced antioxidant defense system (group VI). Thus, although the concentration of H_2O_2 in the homogenate was 12% higher, the concentration of MDA was 5%, and the concentration of DK was 1% higher than in the intact state, it decreased by 8%, 1%, and 1%, respectively, compared to the control group. This indicates that administration of mexidol before ischemia potentiated AMS.

At the 30th minute of the model creation, oxidative stress increased faster due to the expansion of the ischemia zone. Compared to the control group, after mexidol administration, the concentration of H_2O_2 in liver tissue decreased by 2%, MDA concentration by 6%, and DC concentration by 2%, but the concentration of oxidative stress markers was 61%, 40%, and 17% higher, respectively, compared to the intact condition.

Corresponding changes also occurred in the concentration of ADS markers. At the 5th minute of ischemia, the concentration of surface and intrastructural groups of SH proteins increased by 7% each, the concentration of reduced glutathione by 21,5%, and the concentration of TAA by 7% compared with the control group. However, in contrast to these markers, there was no significant change in catalase concentration.

During 30-minute ischemia, the concentration of the surface protein SH group increased by 7%, the concentration of the intrastructural protein SH group by 12%, the concentration of reduced glutathione by 24%, and the concentration of TAA by 11% compared with the control group. The concentration of catalase remained stable (Table 3).

In order to determine the results of reperfusion after amplification of ADS, experiments were conducted on white rats included in the 7th group.

Table 3.

The state of oxidative stress and ADS in the liver tissue in the 24th hour of reperfusion, depending on the duration of ischemia

№	Markers	After 5 minutes of ischemia		After 30 minutes of ischemia	
		M±m	P	M±m	P
1	H_2O_2	3,42±0,21	0,01	4,26±0,09	0,001
2	MDA	2,10±0,19	0,05	2,52±0,09	0,001
3	DK	1,582±0,018	0,001	1,932±0,024	0,001
4	Surface protein SH	31,4±0,8	0,05	23,1±1,0	0,001
5	Structural protein SH	20,4±1,3	*	10,4±1,3	0,001
6	Glutathione	12,1±1,0	*	6,1±0,7	0,001
7	Catalasa	262,9±0,6	0,01	251,6±1,7	0,001
8	TAA	37,8±1,3	0,05	29,4±1,4	0,001

Note: * P>0,05.

It was found that the concentration of H_2O_2 and MDA in liver tissue 15 minutes after the restoration of blood flow on the background of 5-minute ischemia was 2% each, and the concentration of DC was 6,5% higher compared with the intact state. However, as a result of the amplification of the ADS, the OS intensity was greatly reduced compared to the control group. As a manifestation of this, the concentration of H_2O_2 in the liver decreased by 17%, and the concentration of DC and MDA decreased by 11% each. This positive dynamic was also recorded in the following observation days. Thus, in the 30th minute of reperfusion, there was a decrease in the concentration of OS markers in liver tissue. Although the concentration of H_2O_2 in the liver of experimental animals included in this group did not reach the level in the intact state, it decreased by

12% compared with the control group, the concentration of MDA by 21%, and the concentration of DC by 8%. In subsequent periods of observation, the concentration of OS products in the liver tissue continues to decrease, and at 24 h of reperfusion, the concentration of H_2O_2 and MDA in the liver tissue is normalized. The total concentration of DC was 5% higher than the level in the intact state.

Our results show that restoration of blood flow in the liver after 5-minute ischemia as a result of mexidol administration suppresses OS enhancement. Reperfusion after 30 minutes of ischemia produced somewhat different results. At the 15th minute of reperfusion, the H_2O_2 concentration in liver tissue was 24% higher than the intact level, the MDA concentration was 15% higher, and the DC concentration was 11% higher. However, as a result of mexidol exposure, the concentration of the above markers decreased by 27%, 28%, and 11%, respectively, compared to the control group. Such positive dynamics were maintained during the remaining days of the experiment (Table 4).

On the background of a 5-minute ischemia, the concentration of ADS markers in the liver tissue regularly increased from the 15th to the 24th hour of reperfusion.

Compared with the control group in the 15th minute, the concentration of the surface protein SH group increased by 4%, the concentration of the intrastructural protein SH group by 3%, the concentration of the reduced glutathione by 18,5%, the concentration of the catalase by 1%, and the concentration of the TAA by 7%. The increase in the concentration of ADS markers continued in the 30th minute of reperfusion. The concentration of surface protein SH group increased by 3%, the concentration of structural protein SH group increased by 2%, and the concentration of reduced glutathione and TAA increased by 7% each. The concentration of catalase remained stable.

In the 24th hour of reperfusion, the concentration of surface protein SH group in liver tissue increased by 3%, the concentration of structural protein SH group by 8%, the concentration of reduced glutathione by 11%, the concentration of catalase by 1%, and the concentration of TAA by 9% compared with the control group. This illustrates how the ADS recovery rate became wave-like as the reperfusion duration increased (Table 4).

Table 4.

The effect of reperfusion on OS and ADS 30 minutes after ischemia on white rats taken mexidol

№	Markers	Intact state	After reperfusion				
			15 min	30 min	1 hour	3 hour	24 hour
1	H_2O_2	2,46±0,14	3,04±0,23*	3,26±0,15***	3,67±0,04***	3,44±0,16***	4,28±0,21**
2	MDA	1,55±0,02	1,79±0,07***	1,58±0,15***	2,07±0,13***	2,36±0,17**	3,02±0,20**
3	D K *	1,418±0,011	1,576±0,039	1,594±0,038	1,63±0,055	1,64±0,55	1,856±0,04
4	Surf. protein SH	33,5±0,7	29,4±0,7*	28,8±0,8*	28,7±0,8*	28,6±1,1*	28,8±1,7**
5	Struc. protein SH	22,7±0,7	18,4±1,1*	18,0±1,1*	17,9±1,1*	18,1±1,6**	18,5±2,0**
6	Glutathione	13,2±0,4	10,0±0,9**	9,8±0,7*	9,7±0,7*	9,9±1,0**	10,1±0,8**
7	Catalasa	265,9±0,5	261,2±1,4**	258,2±1,8*	257,6±1,8*	257,0±2,2*	257,2±2,3*
8	TAA	40,6±0,3	36,9±0,7*	35,7±1,0*	34,6±0,9***	34,4±1,4*	34,8±1,8**

Note: * P< 0,01; ** P< 0,05; ***P <0,001

The increase in the concentration of ADS markers in the liver during reperfusion after 30-minute ischemia has slightly different results than after 5-minute ischemia.

At the 15th minute of reperfusion, the consentration of surface SH group protein was 10% higher than in the control group. The increase in the consentration of intrastructural SH group proteins reached 19%. Compared with the control group, the concentration of reduced glutathione increased by 21%, the concentration of catalase increased by 2%, and the concentration of TAA increased by 4%. This increase continued in the following days of experiment. At the end of the experiments (at the 24th hour of reperfusion), the concentration of ADS markers was closer to normal. The concentration of surface SH-group protein was 14% lower than normal, the concentration of intrastructural SH-group protein was 18% lower than normal, the concentration of reduced glutathione was 24% lower than normal, the concentration of catalase was 3% lower than normal, and the concentration of TAA was 14% lower than normal.

After the reperfusion on the background of ischemia, which is caused by the strengthening of ADS in the body, relevant changes have also occurred in the structure of the liver.

Dystrophic and degenerative changes of hepatocytes were significantly reduced in the 30th minute of reperfusion on the background of 5-minute ischemia. Dilatation of the center of the sinusoids and other signs indicate a relative improvement in the blood supply to the liver.

3 hours after reperfusion, signs of improvement in the regenerative recovery process in the liver, including the phenomenon of "phenomenon of signs of dark cytoplasm," enlargement of the sinusoids, and the plethora of the central vein, are observed. This process is more pronounced after 24 hours. However, as in previous studies, the positive changes in liver structure are inversely proportional to the duration of ischemia. However, after reperfusion in animals with 30-minute ischemia, the regenerative process in the liver is slightly weakened. Only after the first hour of reperfusion do dystrophic changes in hepatocytes begin to reverse. At 3 hours of reperfusion, hepatocytes show signs of rapid regenerative processes such as enlargement of nuclei, thickening of cytoplasm, and restoration of cell membranes. All this proves once again that there is a correlation between oxidative stress in the liver during ischemia or reperfusion and structural changes in liver tissue. In this regard, it is crucial to administer an antioxidant to the ischemic organ as soon as the blood flow ceases.

RESULTS

1. In parallel to hepatic artery compression, oxidative stress develops in the liver tissue, although the cellular structure of the liver is preserved, the integrity of hepatocytes around the portal tract and central vein increases, and hyaline-droplet and hydropic dystrophies develop. The main destructive effect on the cell membrane is exerted by hydrogen peroxide [1, 10, 11].
2. In reperfusion syndrome, which develops as a result of blood flow restoration in the liver, the intensity of oxidative stress depends on the duration of ischemia. At the 15th minute of reperfusion performed on the background of 5-minute ischemia, the H_2O_2 concentration in the liver tissue increased by 28.5%, the MDA concentration by 15%, and the and the DC concentration by 5.5%. On the background of ischemia lasting 15 minutes, the increase in the concentration of oxidative stress markers at the 15th minute of reperfusion amounted to 49%, 22%, and 21%, respectively [4, 6, 9].
3. FLP in liver tissue in reperfusion syndrome is directly proportional to the duration of reperfusion. At the 15th minute of reperfusion after 5-minute ischemia in liver tissue compared to the intact state, the concentration of H_2O_2 increased by 28.5%, the concentration of MDA by 15%, and the concentration of DC by 5.5%. At the 30th minute, these increases were 54.5%, 51%, and 9%, respectively. At the 60th minute, although H_2O_2 concentration did not change, MDA concentration increased by 57% and DC concentration increased by 13% [2, 3, 14].
4. Since reperfusion after 15-minute ischemia intensifies free lipid peroxidation in liver tissue, at the 30th minute of reperfusion, compared with the intact state, the concentration of H_2O_2 in the homogenate increased by 62%, the concentration of MDA by 51%, and the concentration of DC by 26%. At 60 minutes, these increases were 66%, 83%, and 29%, respectively. At 3 hours after reperfusion, the H_2O_2 concentration in the homogenate prepared from liver tissue increased to 82%, the MDA concentration to 119%, and the DA concentration to 32% [5, 9, 14].

5. During reperfusion after 30-minute ischemia, the FLP process in liver tissue became more intensive. At the 15th minute of reperfusion, compared to the intact state, the concentration of H_2O_2 increased by 68%, the concentration of MDA by 61%, and the concentration of DC by 25%. A correlation between the duration of reperfusion and the intensity of the FLP process was established; the difference in the increase in H_2O_2 concentration after 24 hours was 73%, the difference between MDA concentrations was 62%, and the difference between DC concentrations was 36% [3, 6, 8].
6. Strengthening the antioxidant defense system significantly prevents oxidative stress during ischemia. However, as the duration of ischemia increases, the probability of oxidative stress increases. At the 5th minute of ischemia, H_2O_2 concentration in liver tissue remained normal in 80%, MDA concentration in 60%, and DA concentration in 40% of experimental animals. In ischemized liver tissue under the influence of Mexidol the general antioxidant defense system (ADS) of the organism increased. However, as the duration of ischemia increases, the ADS tends to weaken [5,7,12,13].

PRACTICAL RECOMMENDATION

1. To prevent reperfusion syndrome in liver transplantation, it is important to administer antioxidants to increase the total antioxidant defense of the body from the moment of ischemia to the restoration of blood flow and after that (up to the 24th hour of reperfusion).
2. Pathologists, hepatologists, surgeons, biochemists, and pharmacologists can use the results obtained with antioxidants to prevent reperfusion syndrome.
3. The obtained information can be used in the educational process, i.e. in the classes of pathological anatomy, pathological physiology, biological chemistry, pharmacology, surgical diseases, as well as in the training of doctors and residents in the relevant specialties.
4. The information obtained from our research in reperfusion syndrome can be reflected in monographs and information on liver diseases and transplantology.

List of published scientific articles on the topic of dissertation

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LIST OF ABBREVIATIONS

FLP - Free Lipid Peroxidation
ADS - Antioxidant Defense System
MDA - Malondialdehyde
DC - Diene Conjugates
SH - Sulfohydryl
TAA - Total Antioxidant Activity

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