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METABOLIC CHANGES IN LYMPHOCYTES, NEUTROPHILS AND PLATELETS DURING ISCHEMIC-REPERFUSION INJURY OF THE LIVER

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ABSTRACT

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GENERAL CHARACTERISTIC OF WORK

Relevance of the topic and degree of elaboration. According to the World Health Organization, a third of the elderly population of the planet suffers from one or another liver disease. Currently, transplantation and partial resection of the liver are widely used in the world practice in the radical treatment of severe liver damage and diffuse diseases of various etiologies[1].

Although liver transplantation is a progressive treatment method based on new technologies, it is accompanied by a number of complications[2]. Reperfusion syndrome is more important among these complications. Since ischemia occurs after the transplanted part of the liver is taken from the donor, a number of toxic metabolites are formed there[3]. Ischemia-reperfusion syndrome, which develops after restoration of blood circulation, causes liver failure in the early postoperative stage.

It has been established that ischemia-reperfusion injury (IRI) of the liver consists of immunological and morphological changes and represents a complex pathological and adaptive reaction of the body[4]. The main pathochemical mechanism for the development of IRI in the liver is oxidative stress[5]. The imbalance between tissue oxygen

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demand and incoming oxygen, as well as a sharp increase in its partial pressure in liver cells after restoration of blood flow, create favorable conditions for the formation of free radicals (superoxide anion, hydrogen peroxide, hydroxide radicals). The latter, in turn, causes activation of the process of lipid peroxidation and, finally, a change in the cellular and subcellular membrane structure of hepatocytes[6,7]. At the same time, it has been established that all conditions that cause a violation of the ratio between the delivery of oxygen to the tissues and the demand for it can cause ischemic damage to the liver - hypoxic hepatitis (HH)[8].

As a result of an increase in oxygen radicals during IRI in the liver, first of all, the intracellular energy potential in the body decreases. At the same time, it is known that the liver, which has a rich complex of mitochondria, plays an important role in the formation of energy balance and regulation of bioenergetic processes in the body[9]. İn IRI as a result of microcirculatory, structural and metabolic changes, acceleration of the process of apoptosis and necrosis of hepatocytes, are disrupted many functions in the liver and liver failure occurs[10,11].

In addition to all this, a number of processes occurring in the body during IRI in the liver, especially changes in blood cells, have not been investigated. İt is also known that blood cells, as an element

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of the internal environment, participate in all physiological and pathological processes of the body[12,13] and therefore the metabolic changes occurring in lymphocytes, neutrophils and platelets during IRI are an important pathogenetic link in liver transplantation and are important to study.

The object and subject of the research. The object of the study was 90 white mongrel rats, kept in vivarium conditions at the Scientific Research Center of Azerbaijan Medical University.

The subject of the study was the study of cytochemical changes of lymphocytes, neutrophils and thrombocytes in the blood of intact animals, after modeling hipoxyc hepatites (HH), HH in the background of created ischemia of varying duration and HH in the background of created ischemia of varying duration and folowed reperfusion.

The purpose of the research: Is to study of changes in the cytochemical status of lymphocytes, neutrophils and platelets in IRI of the liver depending on the duration of ischemia created against the background of the HH model.

The Tasks of the Research:

1. To study cytochemical parameters in peripheral blood cells (lymphocyte, neutrophil, platelet) of intact animals.
2. To study the cytochemical status of lymphocytes, neutrophils and platelets in the blood of animals with HH model.
3. To study changes in the cytochemical parameters of lymphocytes, neutrophils and platelets after ischemia was created against the background of the HH model depending on the duration of ischemia.
4. To determine the effect of reperfusion on the metabolic parameters of lymphocytes, neutrophils and platelets depending on

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[13] Circu, M.L. Reactive oxygen species, cellular redox systems, and apoptosis/ M.L.Circu, T.Y.Aw.// Free Radical Biology and Medicine, -2010. 48(6), -p.749-762.

the duration of ischemia against the background of the HH model.

5. To conduct a comparative analysis of changes in the cytochemical parameters of lymphocytes, neutrophils and platelets in the blood of animals with ischemia and reperfusion against the background of the HH model.

Research methods: The concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) enzymes and the level of total bilirubin (TB) were determined by biochemical analysis methods in the blood samples taken from experimental animals.

Metabolik chances in lymphocytes, neutrophils and and thrombocytes - succinate dehydrogenase (SDG), nicotinamide-adenine-dinucleotide phosphate oxidase (NADPHO), myeloperoxidase (MPO), acid phosphatase (AcPH), alkaline phosphatase (AlPH), glycogen (GL) and phospholipids (PHL) were determined by citochemical methodes.

SPSS-22.0 package programs were used for statistical calculations of quantitative indicators obtained from experiments.

The main provisions defended:

1. Metabolic changes in lymphocytes, neutrophils and platelets during ischemic reperfusion, which occur in the liver against the background of HH, appear depending on the duration of the pathological process and are accompanied by depression of mitochondrial and lysosomal enzymes after prolonged ischemia.

2. Changes in mitochondrial and lysosomal enzymes during ischemic reperfusion that occur in the liver against the background of HH have different directions and phase character depending on the duration of ischemia and reperfusion.

3. During ischemic reperfusion in the liver against the background of HH, the strength and direction of intracellular and extracellular correlations in lymphocytes, neutrophils and platelets changes depend on the duration of ischemia.

Scientific novelty of the research: İn this research comprehensively studied intracellular metabolic changes in lymphocytes, neutrophils and platelets against the background of

HH, after HH+ischemia of various durations and HH+ ischemia of various durations with folowed reperfusion.

It has been established that changes in cytochemical parameters in blood cells as a result of İRİ of the liver have different directions and there is a correlation between the occurring changes and the duration of ischemia. It has been established that intracellular and extracellular changes in lymphocytes, neutrophils and platelets has a phase character and depend on the of duration ischemia.

More informative criteria based on changes in the cytochemical parameters of lymphocytes, neutrophils and platelets ın the post-ischemic-reperfusion period of the liver have been determined for diagnosing İRİ of the liver.

Theoretical and practical significance of the research: In experiments conducted on rats, it was established that the HH and the ischemia-reperfusion created on its basis cause a serious change in the function of blood-forming elements -neutrophils, lymphocytes and platelets. It has been found that there is a fair and strong correlation between the enzymes SDG and NADPHO, as well as between GL and PHL. At the same time, it was shown that, thanks to these correlations, these enzymes cause serious changes in the structure of the liver, inducing each other. All this sheds new light on the mechanism of liver damage during ischemia-reperfusion. The obtained results are not only a new approach to the pathogenesis of the destructive effect IRI of the liver, but also created the theoretical basis for adding drugs that serve to normalize the level of the mentioned enzymes to IRI treatment plan.

Metabolic disorders developed as a result of hypoxia and ischemia in the part taken for transplantation in the donor liver are detected in time with the complex cytochemical examinations performed in the blood, are a theoretical basis for the prevention of possible will developed complications. More informative criteria based on changes in the cytochemical indicators of lymphocytes, neutrophils, and platelets have been determined for the diagnosis IRI of the liver in the postischemic-reperfusion period.

Approbation and application: The dissertation materials were discussed at the following scientific meetings:

1. 1st İnternational Rumi Pediatric Congress and 3rd project Congress. Konya, Turkey, 4-7 December, 2019.

2. Uluslararası Bilimsel araştırmalar Konqresi, Adana. 21-23 şubat, 2020.

3. 8th İnternational Cevher Nesibe Health Science Conference, 19-20 november, 2021, İstanbul, Turkey.

4. 5th İnternational New York Confrans on Evolving Trends in İnterdisciplinary Research Practices held and April 3-5, 2022/Manhattan, New York City.

5. Middle East İnternational Conference on contemporary scintific studies-VII, 3-4 March 2022, Beirut Arab University, Lebonon.

6. 5th İnternational African Conference on Current Studies of Scienc. Technology Sosial Sciences 2-5 february, 2022, Cairo, Egypt.

7. 5th İnternational New York Acedemic Research Congress, 23-24 April, 2022.

8. ATU-nun ETM-nin Elmi-Metodik Şurasında. Bakı, 17.03.2022.

In total 15 scientific works on the topic of the dissertation - 7 articles, 2 of which published abroad, 8 reports and theses, 5 of of them abroad, were discussed at republican and international conferences.

The name of the institution where the dissertation was perfomed: The dissertation work was carried out at the Scientific-Research Center of the Azerbaijan Medical University**.**

Scope and structure of the dissertation: The dissertation consists of 178 pages compiled on a computer (284.433 characters), an annotated introduction (9.480 characters), Chapter I of the literature review (59.424 characters), Chapter II of materials and methods (18.520 characters), 3 chapters of personal research (63.445 + 55.797 + 12.724 characters) Conclusion (22.867 characters), Results (1.594 characters), Practical recommendations (674 characters), list of references (35.617 characters). The bibliography includes 196 sources, of which 4 are in Azerbaijani, 27 in Russian and 165 in other languages. The dissertation work is illustrated with 23 tables and 23 figures.

MATERİALS AND METHODS OF RESEARCH

The research work was carried on 90 mongrel rats, weighing 180-200 grams. The experiments were carried out in accordance with the regulations EEC 86/09 and LINESCO (Paris) adopted by the European Society of Bioethics. Three models were created on experimental animals. One of them is the hipoxic hepatites (HH) model, the other is an ischemia model created against the background of HH, and the third is an ischemia-reperfusion model against the background of HH. For creating the HH model, was used the method of acute blood loss, which was carried out as follows: puncture of the heart of anesthetized experimental animals was carried out along the left parasternal line between the 4th and 5th ribs. A needle with a diameter of 0.3 mm is inserted at a distance of 5 mm dorsally perpendicular to the sternum. Negative pressure is created until blood appears in the syringe. 4-4.5 ml of blood are taken at a rate of 2 ml/100 g/min.

In order to create a model of liver ischemia, 24 hours after creating the HH model, the abdominal cavity of rats under anesthesia was opened through a midline incision and the hepatoduodenal ligament was ligated for 5, 10, 15 minutes (in the corresponding groups). A reperfusion model was created by removing the ligature.
According to the goals and objectives of the research, all experimental animals were divided into 6 groups;

I - control group (5 intact white rats);

II - HH model created a group (15 white rats). It was divided into three subgroups of 5 animals each: A – 5 days after modeling, B – 10 days after modeling, C – 15 days after modeling;

III - a group in which the ischemia of varying duration was created against the background of the HH model (15 white rats). Three subgroups of 5 animals each: A - 5 minutes ischemia, B - 10 minutes ischemia, C - 15 minutes ischemia;

IV - reperfusion was performed after 5 minutes of ischemia against the background of the HH model (15 white rats). Three

subgroups of 5 animals each: A – 5 days after modeling, B – 10 days after modeling, C – 15 days after modeling;

V - reperfusion group (15 white rats) after 10 minutes of ischemia according to the HH model. Three subgroups of 5 animals each: A – 5 days after modeling, B – 10 days after modeling, C – 15 days after modeling;

VI - reperfusion group (15 white rats) after 15 minutes of ischemia according to the HH model. Three subgroups of 5 animals each: A – 5 days after modeling, B – 10 days after modeling, C – 15 days after modeling.

To assess changes in liver and cytochemical parameters of lymphocytes, neutrophils and platelets in blood, the abdominal cavity was opened through a midline incision under full anesthesia and blood was collected from the vena cava inferior. The enzyme activities of ALT, AST and GGT, as well as TB, were determined in serum obtained from the collected blood samples.

Blood smears were prepared to determine the level of SDG NADPHO, MPO, AcPH, AlPH, GL and PHL in the formed elements of the collected blood (lymphocytes, neutrophils and platelets) using cytochemical methods.

SDG and NADPHO in lymphocytes, neutrophils and platelets of experimental animals were determined according to the method of R.P. Narcissov (1970), MPO-V.Yu.Dvornik (1992), AcPH and AlPH - M.K. Shubich (1983), PHL and GL were determined according to the method of McManus and Sheleham-story (1983). The results of cytochemical reactions were quantified by the number of granules per cell (50 cells) in the enzymes SDG and NADPHO (g/l, g/n, g/tr), and in MPO, AcPH, AlPH, GL and PHL the average cytochemical indicator per 100 cells is determined by the mean cytochemical index indicator (mci). MCI was determined using the Kaplow formula.

All numerical indicators obtained during the research were statistically analyzed taking into account modern recommendations. During statistical processing of cytochemical parameters, the coefficients of variation (V), asymmetry (A) and excess (E), as well as the average cytochemical index (M±m), characterizing the

distribution of SDG, NADPHO, MPO, AcPH, AlPH, GL and PHL were determined.

RESULTS AND DISCUSSION

Studies have shown that the activity of all three enzymes increased as a result of necrosis of hepatocytes under hypoxic conditions in white rats with a **HH model** (table 1., figure 1.). Thus, compared with the control group, the ALT concentration in subgroup A (after 5 days) increased by 2.6 times (p<001), in subgroup B (after 10 days) by 4 times (p<001), in subgroup C (after 15 days) by 3,1 times (p<001). Compared with the control group, an increase in AST activity was found to be 2.7 times (p<001) in subgroup A, 4.3 times

Table 1. Changes in biochemical indicators of the functional state of the liver after the creation of the HH model.

Indica-tors	Statistical indicators	Control group	Subgroups of group II		
			A (5 days)	A (10days)	A (15days)
ALT, v/l	M±m	134±6,2	342 ± 4,1	536±6,4	420±5,2
	min -max	110-160	301– 386	485-584	390-456
AST, v/l	M±m	142±10,0	385±4,6	606±7,8	510±6,2
	min -max	92-204	326-440)	540-674	460-570
GGT, v/l	M±m	7,2±0,3	45,2±1,4	54,0±1,8	50,6±1,6
	min -max	6,8-8,8	38,1-52,0	46,2-62,0	44,0-56,2
TB, mk mol/l	M±m	2,04±0,12	7,2±0,14	8,08±0,15	6,45±0,12
	min -max	1,56-2,65	6,4-8,0	7,4-8,6	6,01-6,91

Note: All indicators are significantly higher than the control group (p<0.01)

(p<001) in subgroup B, 3.6 times (p<001) in subgroup C. GGT changed significantly (p<0.01) across subgroups and was 6.3 times higher in subgroup A, 7.5 times higher in subgroup B and 7.0 times higher in subgroup C compared to the control group. The level of TB increased by 3.5 times in subgroup A, 3.9 times in subgroup B and 3.3 times in subgroup C.

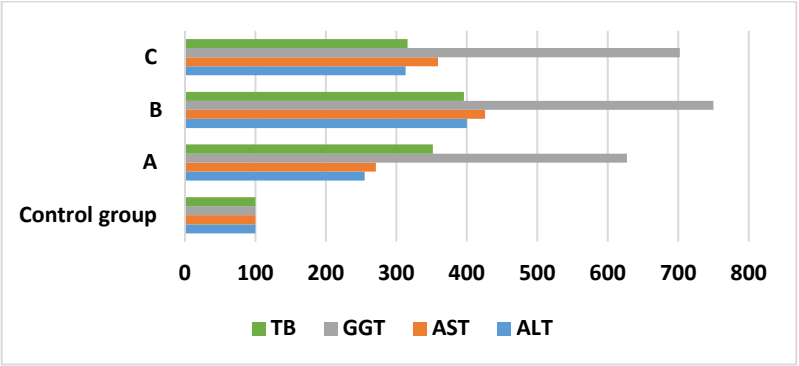

Figure 1.The functional state of the liver after the creation of the HH.

Changes in the cytochemical parameters of lymphocytes, neutrophils and platelets in the blood of white rats after created HH model were as follows. Among the cytochemical parameters of lymphocytes in all three subgroups, the activity of SDG (35.5%; 64.5%; 103.2%), NADPHO significant increase compared to the control group (32.2%; 64.3%; 110.7%) and AcPH (23, 5%; 31.1%; 43.4%). In subgroup C, the activity of mitochondrial enzymes increased more than 2 times. Changes in the same direction were observed at the level of GL (12.5%; 37.5%; 53.1%) and PHL (14.8%; 37%; 57.4%). Changes in the cytochemical parameters of lymphocytes of white rat after created HH model were in the same direction and appeared depending on the duration of the pathological process.

Cytochemical parameters of neutrophils in the blood of white rats with the HH model were high in all subgroups. SDG activity increased by 37.1% in subgroup A, by 64.5% in subgroup B and by 103.9% in subgroup C. Similar patterns were noted in the activity of NADPHO. In subgroups, the activity of this enzyme increased by 38.7%, 73.7% and 117.1%. Corresponding results were also found for AcPH activity (50.0%; 82.4%; 102.9%). In subgroup C, the activity of mitochondrial and lysosomal enzymes (SDG, NADPHO, AcPH) increased. Also activity of MPO (8.0%; 13.6%; 20.9%),

AlPH (15.9%; 22.1%; 30.3%), GL (14.7%; 26.5%; 41 .2%) and PHL (21.8%; 28.2%; 45.5%) increased.

SDG activity in platelets increased by only 20.0% in subgroup A, by 65.0% in subgroup B and by 80.0% in subgroup C. The same level of results was noted for NADPHO activity: NADPHO activity increased by 22.2 %, 55.6%, 88.9% respectively to the A,B,C subgroups. Also, AcPH activity increased by 34.1%, 51.2%, 58.5%. In subgroup C, the activity of mitochondrial and lysosomal enzymes (SDG, NADPHO, AcPH) was higher than in the control group. At the same time, AlPH varied from 33.3% to 88.9%. GL activity compared with the control group, in the corresponding subgroups was 25.0%, 58.3%, 70.8%; PHL activity was 17.4%, 41.3% and 56.5% higher in the respective subgroups.

Thus, serious, complex and multicomponent (mitochondrial, lysosomal, peroxidase-somal) changes occur in the intracellular metabolism of the shaped elements of blood of rats with the HH model.

At the next stage of research, biochemical parameters of the liver and cytochemical parameters of blood cells were studied after **5, 10 and 15 minutes of ischemia against the background of the HH model** (table 1., figure 2.)**..** As a result of research conducted in this group, it was found that after ischemia created under hypoxic

Table 2. Changes in biochemical indicators of the functional state of the liver after various durations of ischemia created on the background of HH.

Indica-tors	Statistical indicators	Control group	Subgroups of group III		
			A (5 min.)	A (10 min)	A (15 min.)
ALT, v/l	M±m	134±6,2	486±5,4	572±6,5	686±7,8
	min -max	110-160	410-530	510-636	614-751
AST, v/l	M±m	142±10,0	524±6,9	734±8,6	786±8,8
	min -max	92-204	456-594	650-812	710-850
GGT, v/l	M±m	7,2±0,3	49,4±1,8	58,0±2,1	58,9±1,9
	min -max	6,8-8,8	43,5-60,0	54,0-65,4	52,5-64,6
TB, mk mol/l	M±m	2,04±0,12	8,0±0,13	8,6±0,14	8,8±0,14
	min -max	1,56-2,65	7,5-8,6	8,0-9,2	8,1-9,4

Note: All indicators are significantly higher than in the control group (p<0.01)

hepatitis conditions, the activity of ALT and AST in subgroup A increased by 3.6 times, and the level of GGT increased by 6.8 times, in subgroup B ALT - 4 times, AST-5 times, GGT increased more than 8 times. In subgroup C, the activity of ALT and AST was 5 times higher, and the level of GGT was 8.2 times higher. The concentration of TB was also higher in all subgroups compared to the control group (3.9 times, 4.2 times and 4.3 times).

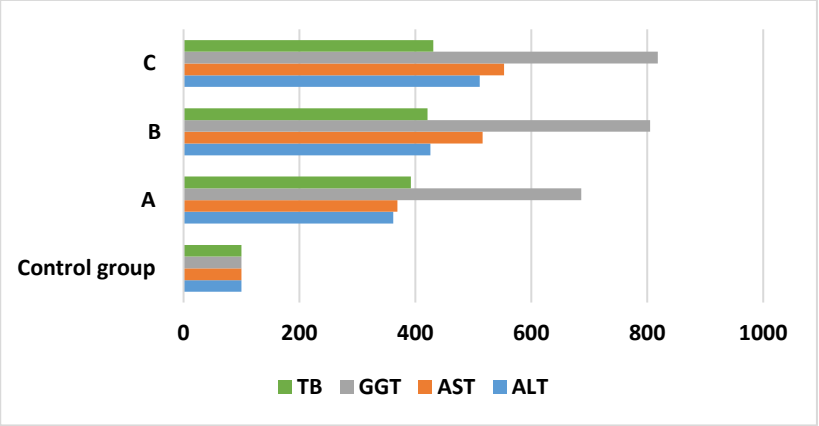

Figure 2.The functional state of the liver after ischemia created on the background of HH.

Changes in the cytochemical parameters of blood cells during ischemia created against the background of HH had different directions (figure 3.). Thus, there was a decrease (respectively in the subgroups) in the dynamics of the activity of SDG (13.0%; 32.3%; 58.1%) and NADPHO (12.5%; 32.1%; 60.8%), AcPH (132,4%-151,0%), GL (143.8% - 206%) and PHL (131.5% - 170.4%) activities had the opposite increase compared with the control group.

In subgroup C, the activity of the enzymes SDG and NADPHO in lymphosytes decreased by 2 times compared to the intact state; such a depressed state of enzymes was observed with serious structural changes. During this time, was noted an increase in AsPH activity and average cytochemical indicators of GL and PHL compared to the intact state.

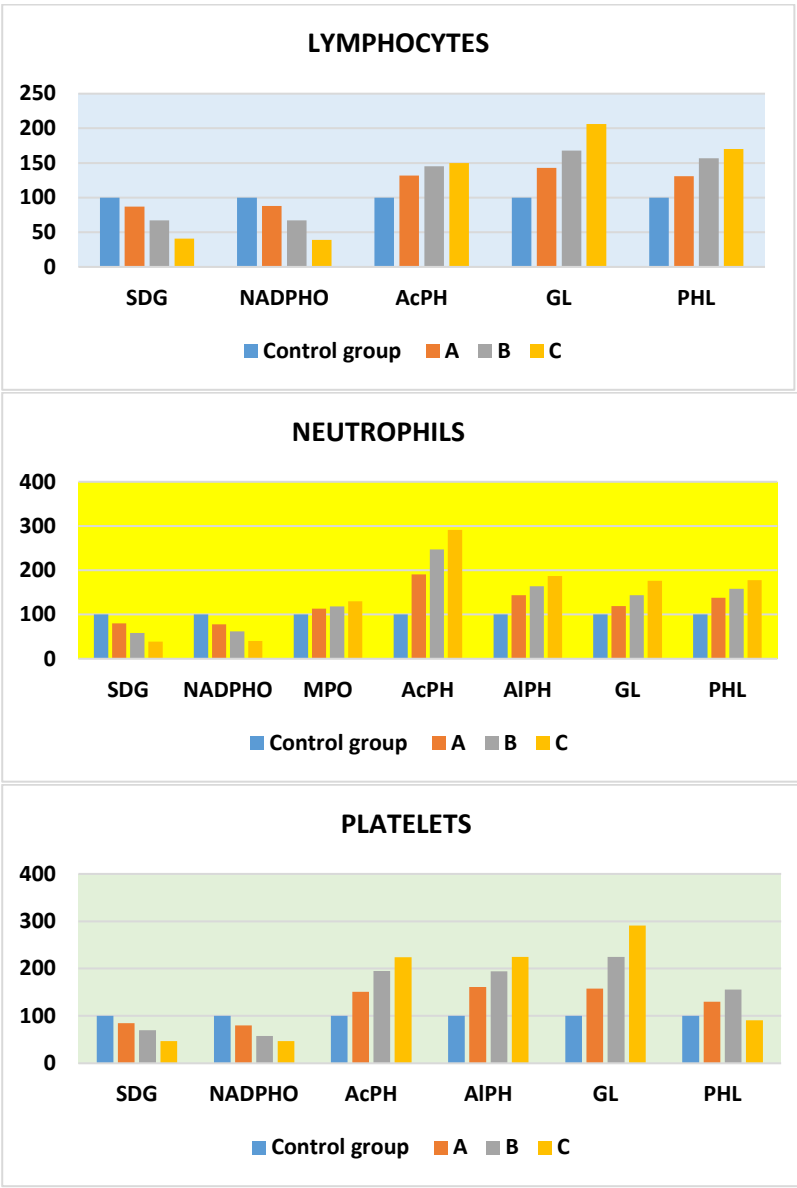

Figure 3. Changes of cytochemical indicators in lymphocytes, neutrophils and platelets after ischemia created on the background of HH.

There was noted a deficiency of lymphocytes with typical AcF activity (negative excess coefficient).

Changes in the activity of neutrophil enzymes were also observed, corresponding to changes in the cytochemical parameters of lymphocytes during ischemia created against the background of HH model. Thus, SDG and NADPHO enzyme activity decreased and were more noticeable in subgroup C. In contrast, in MPO activity was observed a slight increase. These changes in mitochondrial and peroxidase enzymes of neutrophils were observed with high asymmetry (>0.90) and negative kurtosis coefficients (-0.10; -0.16) of structural parameters. In addition to the above-mentioned were observed weakly significant changes in the average cytochemical parameters: the activity of AcPH (191.2%; 247.1%; 191.2%), AlF (144.1%; 164.1%; 187.7%), GL (119.1% -176.5%) and PHL (138.2% -178.2%). These changes were manifested by high asymmetry and negative coefficients of kurtosis of lysosomal enzymes, and it shows a a decrease in neutrophils with typical activity.

Similar patterns were also noted in platelet activity during ischemia created against the background of HH model. Serious changes occurred in the intracellular metabolism of platelets. Thus, there was a decrease in the activity of SDG (15%; 30%; 52.5%) and NADPHO (19.4%; 41.7%; 52.8%), AcPH (151.2%; 195.9%; 224.2%), AlPH (161.1%; 194.4%; 225%), GL (158.3%; 225%; 297.7%) and PHL (130.4%; 156.5%; 191.3%). Changes in the cytochemical indicators of platelets during ischemia created on the background of HH create conditions for changing the numerous functions of these cells.

Thus, changes determined by the cytochemical parameters of circulating lymphocytes, neutrophils and platelets during ischemia created against the background of HH model are assessed as responses to the multiple functions of these cells.

The next series of studies examined the **metabolic changes that occur in all three blood (lymphocytes, neutrophils and platelets) cells in IRI of the liver**. The conducted studies showed

that the average cytochemical parameters of mitochondrial (SDG, NADPHO) and lysosomal (AcPH) enzymes of lymphocytes of the main groups (IV, V, VI) change in different directions. Thus, after short-term ischemia (5 minutes) in group IV, SDG (101.2%; 48.4%; 25.8%) and NADPHO (108.9%; 60.0%; 24.4%) activity was increased, and in groups V and VI there was a sharp decrease ($p<0.01$) after 10 and 15 minutes of ischemia. The noted changes in the activity of SDG and NADPHO ($p<0.01$) have a phase character depending on the duration of ischemia. A significant decrease ($p<0.01$) in AcPH activity in lymphocytes was noted in all subgroups of the three groups of animals with IRI of the liver. In addition, the observed changes in the level of AlPH and PHL in lymphocytes in all three groups with IRI of the liver were unidirectional and increased significantly.

As the duration of ischemia before reperfusion increased, prominent changes in the structural indicators of lymphocytes were observed. More noticeable changes were observed in animals reperfused after 10 minutes of ischemia after 10 days (subgroup B) against the background of high asymmetry in the activity of SDG, NADPHO and AcPH (A-SDG = 1.10; A-NADPHO = 1, 19) a negative excess (E=SDG=-0.30; E-NADPHO=-0.21; E=AcPH=-0.16) was observed. In the group VI changes in the structural parameters taken at different times (after 5, 10, 15 days) in rats reperfused after 15 minutes of ischemia changed more pronouncedly than in group V. It was noted of high variability ($V>56.0$) and asymmetry ($A>1.28$) of NADPHO activity, a deficient-negative excess of cells with typical activity (E NADPHO = -0.22; -0.25;-0.30).

Changes in mitochondrial (SDG), (NADPHO) and lysosomal (AsPH) enzymes of lymphocytes in IRI of the liver have different directions and phase character depending on the duration of ischemia and the period after reperfusion. These changes occur against the background of a relatively high level of GL, which indicates the activation of glycogenesis in the cell.

As a result of the studies, it was established that changes in the average cytochemical indicators of oxidation-reduction enzymes (SDG, NADPHO, MPO) in neutrophils of the main groups (IV, V, VI) occurred in different directions. İn white rats of group IV after short-term ischemia (5 minutes) in subgroups compared with the control group, SDG activity (101.6%; 50.0%; 37.4%) for NADPHO activity (94.3% 40.9%; 35.6%) was increase and in the V and VI groups of rats after 10 and 15 minutes of ischemia there was significant decrease. In addition to all this, in all three groups and subgroups with IRI of the liver, there was a decrease ($p<0.01$) in the activity of MPO and AcPH in neutrophils. The observed changes in MPO activity occur in a time-dependent manner after ischemia and reperfusion: low activity, noted in the average cytochemical parameters in the blood of experimental animals belonging to subgroups A of all three groups increases in the following days and is significantly different from the control group ($p<0.01$). The changes in AcPH activity are unidirectional and decrease depending on the duration ischemia and after reperfusion. Its level is 2 times lower in subgroup C of the V group compared to the control group and 3-3.5 times lower in the all subgroup of the VI group. It should be noted that AcPH is a characteristic lysosomal enzyme for young neutrophilic granulocytes, reflecting the tension of intracellular processes that perform a metabolic function. The observed changes occur after ischemia and reperfusion. During IRI of the liveris noted that the level of AlPH in neutrophils in white rats of group IV is relatively low compared to the control group and higher in the subgroup in groups V and VI, C subgroups of group V and all subgroups of group VI (A,B,C). An increase in the level of GL is probably associated with the activation of gluconeogenesis and synthetic processes in the cell. In IRI of the liver, the level of GL and PHL in neutrophils was higher in the main group of animals compared to the control group ($p<0.01$). Changes in the cytochemical status of neutrophil granulocytes during damage to the IRI create conditions for the adequacy of many functions of these cells.

During IRI, the amount of mitochondrial enzymes (SDG and NADPHO) in platelets gradually decreases across groups. Changes in one direction are observed - a parallel decrease is observed in subgroups B and C of the V group ($p<0.01$), in all three subgroups (A, B, C) of the VI group ($p<0.01$). As the duration of ischemia increases, changes in the structural parameters of the platelet population become more profound. In experimental animals reperfused after 10 minutes of ischemia, after 5 days the variability of SDG was increased 2 times (B-SDG = 70.0), its asymmetry was increased 1.5 times (A-SDG = 0.94), a deficiency of typical active cells - 1.5 times (F-SDG=0.84). Similar changes are observed in the activity of NADPHO. After 15 minutes of ischemia and reperfusion (group V), more pronounced changes in the structural parameters of the platelet population are observed in blood taken at different times (after 5, 10, 15 days) compared to group V. High variability of SDG and NADPHO activity in all subgroups of group VI (A, B, C) (B-SDG≥90.0; B-NADPHO≥70.0), pronounced asymmetry (A-SDG≥1 , 12; A) -NADPHO≥0.96) and deficiency of cells with typical activity (E-SDG≥-0.31; E-NADPHO≥-0.25) increased.

In experimental animals, drastic changes are observed in lysosomal enzymes (AcPH, AlPH), as well as in mitochondrial enzymes, which are the starting enzymes of the respiratory chain. After short-term ischemia, an increase in AcPH and AlPH activity is observed in response to reperfusion. Depending on the time after reperfusion, these indicators gradually increased on 5, 10, 15 days and were higher in blood taken after 15 days (AcPH = 0.61 ± 0.4; AlPH = 0.56 ± 0. 01 osg). A highly significant difference was noted in all subgroups (A, B, C) of reperfused rats (group VI) after 15 minutes of ischemia. Changes, determined in the average typical cytochemical parameters of phosphatases, were observed with high variability in the structure, the predominance of cells with low activity and the presence of single highly hyperactive and low-active platelets. Consistently high phosphatase activity reflects destabilization of the lysosomal membrane and increased catabolic processes in platelets. In addition to mitochondrial and lysosomal

enzymes, sharp changes were noted in the cytochemical parameters of PHL and GL, which play an important role in the formation of platelet structure. Changes in cytochemical levels of PHL and GL were unidirectional and their intensity appeared depending on the duration of ischemia.

Thus, the conducted experiments showed that the final analysis of the cytochemical indicators of lymphocytes, neutrophils and platelets during IRZ of the liver showed that the most serious changes occur after long-term ischemia (figure 4.).

All of the above dictates the importance of analyzing correlations between cytochemical parameters of circulating lymphocytes, neutrophils and platelets in IRI of the liver. Intracellular correlations during IRZ of the liver showed the coordination functional status of various structural components located in the cell and the of complex processes taking place in them.

In studies conducted on experimental animals, the strength, direction and phase character of correlations existing between cytochemical parameters were analyzed. It has been established that the strength and direction of intracellular correlations in lymphocytes, neutrophils and platelets mainly depend on the localization (mitochondrial, lysosomal) and function of cytochemical parameters. Reliable and strong correlations ($r \geq 0.50$) are observed between SDG and NADPHO, between GL and PHL, between AcPH and AlPH. Opposite directions (negative connections) were detected between mitochondrial enzymes (SDG, NADPHO) and lysosomal (AcPH, AlPH) enzymes, as well as between GL and PHL.

The strength of the detected correlations increased depending on the duration of ischemia. After 15 minutes of ischemia, the strength of the correlations increased significantly, a close and highly reliable connection ($r \geq 0.65$; $p < 0.01$) was established between SDG-L and NADPHO-L ($r=0.76$; $p<0. 01$), AcPH and PHL ($r = 0.69$; $p \leq 0.01$), AlPH and PHL–L (r0.70; $p \leq 0.01$). An inverse (negative) correlation was noted between SDG and GL ($r = -0.52$; $p < 0.01$), between SDG and AcPH ($r = - 0.47$; $p < 0.05$).

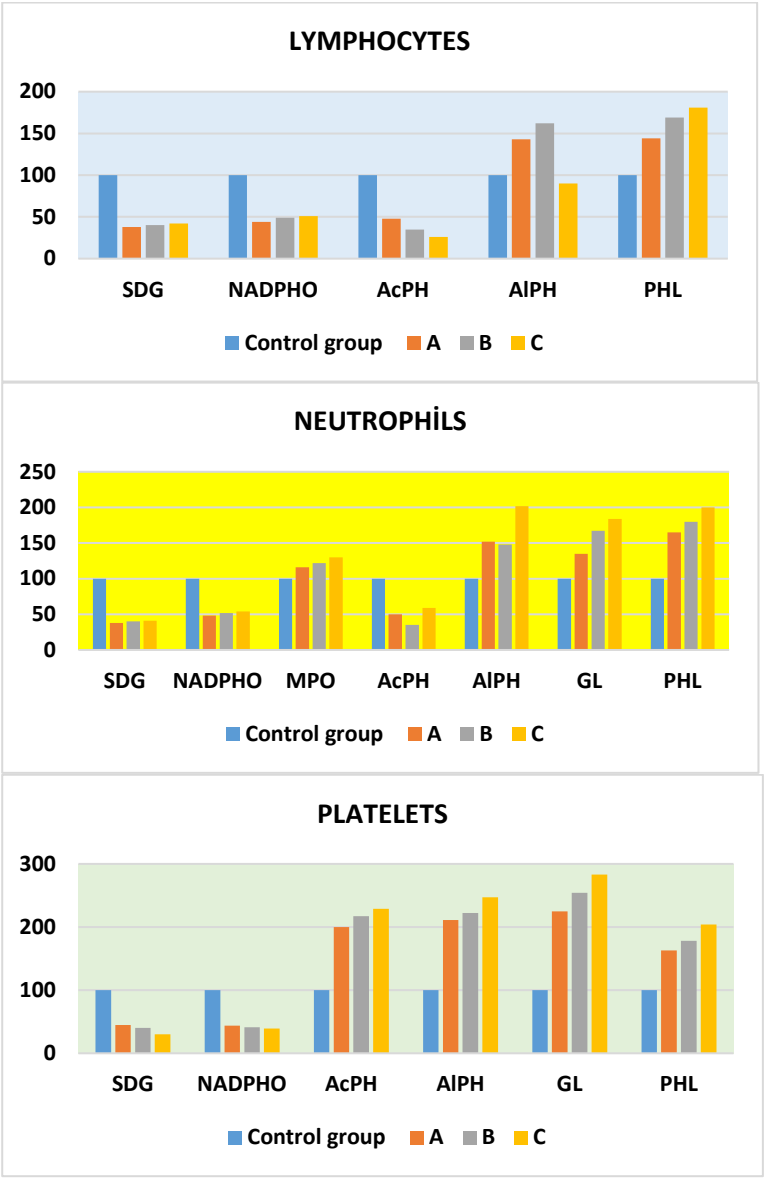

Figure 4. Changes in cytochemical indicators in lymphocytes, neutrophils and platelets in rats reperfused after 15 minutes of ischemia created on the background of HH.

The direction of intracellular correlations in neutrophils was similar to that in lymphocytes and stronger. Intracellular correlations in platelets were not significantly different from those found in lymphocytes and neutrophils.

The final analysis of the materials obtained on the basis of the study allows us to express such an opinion. Changes in the cytochemical parameters of circulating lymphocytes, neutrophils and platelets arter created ischemia against the background of HH are assessed as a response to the ongoing pathological process in accordance with the numerous functions of these cells.

Changes in the cytochemical parameters of blood lymphocytes after created ischemia and reperfusion against the background of HH had different directions. The activity of SDG and NADPHO In blood taken after 15 days decreased by 2 times and such a depressed state of enzymes was observed with serious structural changes in the cells. At this time, there was an increase in AcPH, GL and PHL activity and showed a deficiency of lymphocytes with typical activity.

Changes in the cytochemical indicators of lymphocytes after ischemia-reperfusion created against the background of HH are also observed in neutrophils.Thus, a decrease in the activity of SDG and NADFO enzymes (more noticeable in subgroup C), on the contrary, a slight increase in MPO activity is detected. These changes in mitochondrial and peroxidaseosomal enzymes of neutrophils were accompanied by high asymmetry (>0.90) and negative excess coefficients (-0.20; -0.16) in cell structural indicators. In addition to the above, there was a slight honest increase in the average cytochemical indicators of AlPH, GL and PHL. These changes were manifested by high asymmetry and negative excess coefficients in lysosomal enzymes, which indicate a decrease in neutrophils with typical activity.

Similar patterns were obtained in the activity of platelets after ischemia-reperfusion created against the background of HH and indicate serious changes in the intracellular metabolism of platelets.

Intracellular correlations during IRI of the liver showed the functional state of various structural components located in the cell

and the coordination of complex processes occurring in them (tables 3-5).

Table 3. Correlations between lymphocytes and neutrophils cytochemical indicators (r)

Lymphocytes	Neutrophils						
	SDG	NADPHO	MPO	AcPH	AlPH	GL	PHL
SDG	0,88	0,76	0,65	-0,41	-0,32	-0,35	-0,24
NADPHO	0,76	0,86	0,61	-0,55	-0,52	-0,39	-0,31
AcPH	-0,44	-0,48	0,38	0,84	0,79	0,48	0,49
GL	-0,34	-0,39	0,55	0,64	0,44	0,87	0,68
PHL	-0,30	-0,37	0,48	0,50	0,47	0,70	0.89

Table 4. Correlations between cytochemical parameters of platelets and lymphocytes (r)

Platelets	Lymphocytes				
	SDG	NADPHO	AcPH	GL	PHL
SDG	0,806	0,710	-0,350	-0,301	-0,360
NADPHO	0,710	0,864	-0,310	-0,280	-0,316
AcPH	-0,241	-0,190	0,790	0,480	0,582
GL	-0,316	-0,342	0,490	0,750	0,620
PHL	-0,370	-0,390	0,580	0,672	0,781

Table 5. Correlations between cytochemical parameters of platelets and neutrophils (r)

Platelets	Neutrophils						
	SDG	NADPHO	MPO	AcPH	AlPH	GL	PHL
SDG	0,840	0,750	0,514	-0,385	-0,465	-0,270	-0,190
NADFO	0,750	0,810	0,550	-0,410	-0,402	-0,340	-0,360
AcPH	-0,385	-0,410	0,290	0,792	0,720	0,401	0,410
GL	-0,250	-0,280	0,440	0,580	0,380	0,820	0,610
PHL	-0,210	-0,280	0,390	0,410	0,380	0,610	0,810

The extracellular correlation reflects the functional connections in the cytochemical status of different cells and characterizes the mutual functional connections between individual cells (lymphocyte - neutrophil, lymphocyte - platelet, neutrophil - platelet).

As a result of experiments conducted on white rats, it was established that in IRI of liver cause a serious change in the function of neutrophils, lymphocytes and platelets in the blood. The strength and direction of these changes depend on the localization of cytochemical parameters. There is also a reliable and strong correlation between the enzymes SDG and NADPHO, between GL and PHL. It has been established that, thanks to such correlations, these enzymes cause serious changes in the structure of the liver, inducing each other. All this sheds new light on the mechanism of ischemia-reperfusion injury to the liver. The obtained results represent a new approach to the pathogenesis of the destructive effect of reperfusion syndrome on the liver, and also create a theoretical basis for the inclusion in the treatment regimen of drugs that serve to normalize the concentrations of these enzymes. The completed dissertation work is an experimental study that lays the ground for early detection of metabolic changes in peripheral blood cells during IRZ of the liver and for carrying out treatment measures at an early stage.

Complex cytochemical tests performed in the blood cells can be a theoretical basis for the prevention of possible complications by timely detecting metabolic disorders that develop as a result of ischemia in the part taken for transplantation in the donor liver.

CONCLUSIONS

1. Changes determined in mitochondrial (SDG, NADHO) and lysosomal (AcPH, AlPH) enzymes, also the cytochemical activity of GL of lymphocytes, neutrophils and platelets in hypoxic hepatitis depend on the duration of the pathological process and are in the same direction.
2. Changes in the cytochemical indicators of lymphocytes, neutrophils and platelets in the blood after various durations of ischemia against the background of hypoxic hepatitis have different directions and are manifested depending on the

duration of ischemia: decrease in the activity of SDG, NADPHO, increase activity of AcPH, AlPH, MPO, GL and PHL, a highly significant difference ($p<0.01$) is determined after 15 minutes of ischemia.

3. Changes in mitochondrial (SDG, NADPHO) and lysosomal (AcPH, AlPH) enzymes of lymphocytes, neutrophils and platelets during IRI of the liver have different directions and phase character depending on the duration of ischemia and the period after reperfusion. Changes in the intracellular metabolism of lymphocytes, neutrophils and platelets during IRI of the liver are accompanied by a sharp decrease and depression of SDG, NADHO and AcF after prolonged ischemia.
4. During IRI of the liver, high AlPH activity is observed against the background of high levels of AlPH and PHL in neutrophil granulocytes, which indicates the activation of glycogenesis and synthetic processes in the cell.
5. The strength of intracellular correlations during IRZ in the liver depends on the duration of ischemia. A close and highly reliable relationship ($r\geq0.65$; $p< 0.01$) is determined between SDG and NADPHO, AcPH and PHL, GL and PHL after 15 minutes of ischemia. An inverse reliable relationship exists between SDG and GL, NADPHO and GL, SDG and AcPH. A reliable ($r\geq0.65$; $p<0.01$) extracellular correlation is established between SDG and NADPHO, AcPH and AlPH, PHL and GL, SDG and NADPHO, GL and PHL, PHL and GL.

PRACTICAL RECOMMENDATIONS

1. It is recommended to carry out complex cytochemical examinations for the timely detection of metabolic disorders that develop as a result of IRI of the liver.

2. Complex cytochemical examinations of the blood are a theoretical basis for the prevention of possible complications by timely detecting metabolic disorders that develop as a result of ischemia in the part taken for transplantation in the donor liver.
3. Changes in the cytochemical parameters of lymphocytes, neutrophils and platelets during the postischemic-reperfusion period in the liver are informative criteria for the diagnosis of ischemia-reperfusion syndrome.

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LIST OF ABBREVIATIONS

A- asymmetry coefficient
AcPH-acid phosphatase
AlPH-alkaline phosphatase
ALT-alanine aminotransferase
AST-aspartate aminotransferase
E-excess coefficient
PHL-phospholipid
GGT - gamma-glutamyl transferase
GL-glycogen
HH- hypoxic hepatitis
İRİ-ischemic-reperfusion injury
MPO- myeloperoxidase
NDPHO-nicotinamide-denucleotide-phosphate oxidase
SDG-succinate dehydrogenase
TB -total bilirubin
V- variation coefficient

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