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EXPERIMENTAL ACUTE ISCHEMIC, TOXIC AND ALCOHOL-INDUCED LIVER INJURY: RELATION OF IMMUNOLOGICAL AND METABOLIC PARAMETERS FOLLOWING THE CORRECTION WITH ALLOGENIC HEPATOCYTES

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Abstract
Immune disorders arising from liver damage of various genesis, and the mechanisms of their development have still been poorly studied. The immune system functions are carried out in presence of metabolic processes and their shifts, caused by the action of various agents on the body, and liver cells – hepatocytes as well. Typical metabolic shifts that occur due to the liver damage by various toxic factors are combined with certain features of metabolic disorders in certain organs and tissues, according to the specificity of their structural and functional organization, the nature of the inducing agent and the primary sector of its effect on cells and the body as a whole. The interrelation of numerous metabolic shifts, disorders of the hepatocytes functional activity arising in such a pathology with the immune system dysfunction has not been studied enough yet, and the most effective methods of correction have not been established yet as well. At present the problems of pathogenesis, diagnosis and treatment of acute liver diseases remain the most challenging ones in medicine, both due to the complexity of diagnosis and the choice of optimal treatment methods, and due to the increasing tendency in the number of patients with these diseases. The introduction and development of cellular technologies have created serious scientific prerequisites in this area.

Keywords: correlated relations; liver damage; immune and metabolic disorders.

INTRODUCTION

Taking into account the liver important role in homeostasis regulation and the constant increase in its incidence due to manufacture, everyday life and medicine chemicalization, and abuse of alcohol as well, optimal conditions development for enhancing liver regeneration is particularly true, and the correction of disorders associated with the liver pathology is one of the most important medical and social problems not only in our country, but in all developed and developing countries as well. The functional activity of this very organ significantly affects the state of the immune and endocrine functions, by means of which the regulation of liver regeneration is conducted [1].

There is a large number of works in the literature which are devoted to the correction of liver dysfunctions, by means of cellular technologies in particular, there are isolated studies on erythrocytic disorders and their correction in liver pathology, and in fact there are no works on the corrective effect of allogeneic cells transplantation and their cultural humoral factors on erythrocytes metabolic activity [2].

Hepatocytes have become the first type of cells used for clinical purposes - cell therapy for patients with congenital and acquired liver pathology. In contrast to precursor cells and stem cells, hepatocytes cultures have a very limited capacity for division, which is a serious limiting factor for their practical use, but under stressful conditions (including acute liver damage) they fall into a state of hyperplasia and acquire their ability to actively reproduce. This property of hepatocytes has provided the basis for their use in recovery processes of liver diseases [3].

The effectiveness in replacing cellular liver defects in congenital and acquired diseases, the ability to stimulate the

organ's own regeneration, the absence of the dangers of fibrosis development, depend mainly on the cells used. A number of studies have shown that under certain cultivation conditions various types of cells are capable of expressing markers specific to hepatocytes. However, the real functionality of certain cells remains unproven; therefore, studies devoted to the metabolic activity of allografts are urgently needed [4].

The relation of numerous metabolic and immunological changes and disorders in the functional activity of hepatocytes, arising due to various liver pathologies, with the function of the immune system destabilization has not been sufficiently studied yet, and the most effective methods of pharmacological correction have not been established as well [5].

In reliance on the above-mentioned facts, the aim of the study is to determine the relationship between indicators of metabolic and immune status in liver damage by means of various pathogenic factors following correction with allogeneic hepatocytes.

MATERIALS AND METHODS.

The studies were carried out on 96 male Wistar Rats weighing 100-160 g. In the experiments the animals that had undergone the quarantine treatment in Kursk State Medical University vivarium and had not shown external signs of any diseases were used. All the studies were performed at the same period of time, from 8 am to 12, in compliance with the principles stated in the Convention for the Protection of Vertebrate Animals used for Experimental and Other Purposes (Strasbourg, France, 1986) and in accordance with the rules of good laboratory practice of the Russian Federation (Decree of the Ministry of Health of the Russian Federation No. 267 dated June 19, 2003).

Acute intoxication with carbon tetrachloride (CCl_4) in laboratory animals was carried out by its intramuscular administration at a dose of 3 ml/kg in 50% solution in olive oil five times with an interval of 24 hours [6].

Acute ischemic liver injury (AILI) was modeled by an operative approach. For this purpose, one hexenalum intraperitoneal anesthesia was used for each animal unit at a dose of 30 mg/kg of body weight. For the operative approach, a supramedian laparotomy was chosen. The liver injury was caused by clamping lig. hepatoduodenale with the tourniquet for 20 minutes. Lig. hepatoduodenale infiltration was produced with 0.5 ml of 0.5% novocain solution, which was performed prior to clamping. Suture material was removed from lig. hepatoduodenale after 20-minutes occlusion. The operating wound was sutured through all the panniculus in layers, then it was treated with 2% iodine solution and abacterial gauze bandage with an antiseptic was applied [7].Alcohol intoxication was simulated by forced intragastric administration of 20% ethanol solution at a dose of 2 ml/kg (2.92 g/kg) in 24 hours for 60 days [8].

In experimental animals, blood sampling for research was carried out under anesthesia, by the method of intra-cardiac injection. Plasma and erythrocytes were obtained from heparinized blood by centrifugation for 5 minutes at 400g.

The assessment of immunological reactivity was based on the indicators of B-cells response (the number of antibody-generating cells - AGC) and delayed hyperresponsiveness (DHR) (the mass difference in the local and contralateral lymph nodes – MD and the difference in the number of karyocytes in them - KD) [9].

With the help of a specialized locally-produces commercial kit “TBK-Agat” (“Agat-Med” Russia), and using a foreign spectrophotometer “Apel-330” (Japan) at a specific wavelength of 535 nm and 570 nm we evaluated and studied the intensity of lipid peroxidation processes. These processes were determined by the content of two indicators in erythrocytes and blood plasma, i.e. malonyldialdehyde (MDA) and acylhydroperoxides (AHP). The state of the antioxidant system of the body was assessed by direct/competitive enzyme-linked immunosorbent assay (ELISA) with the detection of reaction products in the wavelength range 405-630 by means of commercial kits. By the method based on the ability to inhibit ascorbate- and ferro-induced oxidation of tween-80 to MDA we assessed the total antioxidant activity (TAA) and superoxide dismutase activity (SOD), “Bender Medsystems” (Austria). The level of nitrogen oxide stable metabolites (SM_{NO}) was determined by two analytical operations: measurement of endogenous nitrite and conversion of nitrate into nitrite using nitrite-reductase, followed by total nitrite determination by the absorption of azo dye in the Griess reaction at a wavelength of 540 nm using a commercial kit for solid-phase ELISA "R&D" (England). Recording and registration of EIA (enzyme immunoassay) results were performed strongly by means of one automatic reader for EIA, the product of domestic-owned firm - Efos 9305 (Russia) [10].

Neutrophils were obtained on velocity sedimentation gradient of ficoll-urografin (p = 1.078) from the blood taken. Neutrophil phagocytic activity was assessed in peripheral blood; it was fulfilled by phagocytic number, phagocytic index, index of phagocyte activity (PN, PI, IPA). Oxygen-dependent activity of neutrophils was assessed by NBT-spontaneous (NBT-sp.) and NBT-stimulated with non-opsonized and opsonized zymosan (NBT-st. no/z, NBT-st. o/z) tests, coefficients of opsonization, activation to unopsonized and opsonized zymosan (CO, CAno, CAo) [11].

For a comprehensive assessment of drugs effectiveness and histomorphologic confirmation of pathological processes being modeled, a histological examination of the liver was performed.

Isolation of allogeneic hepatocytes (AH) from the animals in 5-6 days after their birth was carried out according to M.N. Berry, D.S. Friend method, for this purpose, the liver was crushed after its harvesting, hepatocytes were extracted from the tissue by extrusion using a glass homogenizer in medium 199. The obtained cell suspension was washed twice by centrifugation for 10 min at 400 g, diluted in medium 199, and the number of cells was counted. Their viability was determined in the test with trypan blue, while in further experiments, cell suspensions containing more than 90% of viable cells were used. After concentration by centrifugation, a pool of cell suspension from 2-3 rats or 3-4 mice at a concentration of 2 x 10^6/kg was immediately intraperitoneally injected ten times in 24 hours, at a volume of 0.5 ml in medium 199. During all the manipulations with cell suspension, the temperature of the used medium 199 was 36-37 ^oC.

Statistical processing of the research results was carried out according to the generally accepted criteria of the variable-based statistical analysis with the calculation of mean values (M), arithmetic mean error (m) using the software package Microsoft Excel, 2010. The significance of differences was assessed by U-test. Differences with $p < 0.05$ were considered statistically significant.

RESULTS AND THEIR DISCUSSION.

Forced intake of ethanol resulted in the development of LPO processes (an increase in the concentration of MDA and AHP), a decrease in antioxidant defense factors (a decrease in TAA and catalase activity, SOD). In addition, a decrease

in the level of SM_{NO} was revealed. The introduction of AH normalized the TAA, corrected the content of LPO products and the activity of antioxidant defense enzymes, normalized the activity of catalase, and brought the concentration of MDA, AHP and SM_{NO} closer to the normal parameters. When assessing the indicators of erythrocytes functional activity in circulating blood during alcohol intoxication, an increase in LPO products (MDA, AHP), a decrease in the activity of antioxidant defense enzymes (SOD, catalase), SM_{NO}, and sorption capacity of the red blood cells membrane (SCE, SCG) were noticed. The use of AH normalized the sorption capacity of the glycocalyx, brought the levels of MDA, SM_{NO}, catalase to normal values, corrected AHP and the sorption capacity of erythrocytes. As for the functional and metabolic activity of peripheral blood neutrophils, a decrease in their phagocytic ability (decrease in PI, PN, IPA) was noted upon activation of oxygen-dependent metabolism (increase in NBT-spontaneous and stimulated by zymosan and PAN). The use of AH normalized the PN, PAN, corrected PI and IPA towards the control level. In addition to the above mentioned, the delivery of AH stabilized the NBT-st. and corrected NBT-sp.

Acute ischemic liver injury also caused an increase in the level of lipid peroxidation products, MDA and AHP in particular, with a simultaneous increase in catalase activity. The results obtained indicate pronounced changes in the immune reactivity and functional activity of polymorphonuclear leukocytes under the conditions of ischemic liver injury. The use of allogeneic hepatocytes in the animals with ILI (ischemic liver injury) made it possible to normalize the PN of polymorphonuclear leukocytes and their oxygen-dependent activity, to correct, but not to the normal level, the number of AGC and PI of peripheral blood neutrophils.

When evaluating oxidative parameters of the blood plasma of experimental animals with ATLI (acute toxic liver injury), the activation of LPO processes (an increase in the level of MDA and AHP), a decrease in the indicators of antioxidant protection (TAA, the activity of SOD and catalase) and the content of SM_{NO} were established. What is more, an increase in all the studied parameters of the functional and metabolic activity of peripheral blood neutrophils was recorded, i.e, oxygen-dependent (increased NBT-sp., NBT-st. no/z, NBT-st. o/z, CAno, CA_O, CO) and phagocytic activity (increase in PI, PN and IPA). The introduction of AH corrects the metabolic parameters of blood plasma (the concentration of MDA is an exception), in erythrocytes it normalizes the content of TAA, AHP and corrects SCG and the activity of antioxidant enzymes (SOD and catalase).

The pathogenesis of the revealed disorders can be explained based on the idea about the interdependence of different links supporting the constancy of the internal environment of the body. For this purpose, we analyzed the matrix of multiple correlation between the components of metabolic and immunological statuses, the results of which are presented in the tables. A correlation analysis between indicators of metabolic status at the local and systemic levels in acute tetrachloromethane, ischemic and alcoholic liver injury after the therapy with allogeneic hepatocytes was carried out as well.

Correlation analysis was used to determine the presence of reliable connections between different systems of immunological and metabolic parameters: immune status, metabolic status in blood plasma and erythrocytes depending on patients' group.

Analyzing the matrix of Spearman's multiple correlation between the indicators of metabolic and immune status in ischemic liver injury at the systemic level after correction with allogeneic hepatocytes, we have established 9 significant strong direct and reverse correlations. In such a case, the dynamics of MDA and SOD is associated with changes in two indicators of the immune status: MDA with MD and PI, and SOD with CAo and PN. Most of the strong links were found between catalase and indicators of the immune status AGC, NBT-sp., NBT-st. o/z and CA_{no} (Table 1).

Having analyzed the matrix of Spearman's multiple correlation between the indicators of metabolic and immune status in acute liver ischemia after correction by allogeneic hepatocytes at the local level, 12 significant strong correlation relations were established. In this respect, the largest number of relationships was determined between AGC and the following indicators of metabolic status: MDA, TAA, SOD, catalase, and SCE. The indicator of SCE itself correlated with three indicators of the immune status: AGC, NBT-sp, CO. The rest of the indicators had one or two strong relationships (Table 2).

Comparing the indicators obtained while setting the Spearman's matrix between the values of the metabolic status at the systemic and local levels after correction with allogeneic hepatocytes, a number of relationships was also revealed. Six correlations were established, 2 links for the local level of catalase (TAA, SCE), 2 links for the local level of SM_{ON} (SOD, catalase). One relationship at the local level for MDA with TAA and TAA with SCG (Table 3). Furthermore, we also carried out a similar correlation analysis, but in toxic liver damage after correction with allogeneic hepatocytes. Analyzing the matrix of Spearman's multiple correlation between the indicators of metabolic and immune status in toxic liver damage at the systemic level, we revealed 7 strong direct and inverse reliable correlations. At the same time, 2 bonds each for the catalase indicator (AGCK, NBT-st. no/z), TAA (CAno, CO), KD (MDA, SOD), CAno (TAA, SM_{ON}). The strongest direct relationship was noted between CAno and SM_{ON} (Table 4).

Analyzing the matrix of Spearman's multiple correlation between the studied parameters at the local level after correction with allogeneic hepatocytes, 12 reliable relationships were established, while the dynamics of the metabolic parameters of MDA and AHP is associated with 3 different indicators of the immune status, and the level of SCG with two indicators (AGC, IPA). The indicator of AGC immune status was also connected with direct and inverse association with the indicators of metabolic status (MDA, SOD SCE) (Table 5).

Comparing the indicators obtained while compiling the Spearman's matrix between the values of the metabolic status

at the systemic and local levels following the correction with allogeneic hepatocytes, 6 significant correlations were also revealed. Catalase at the systemic level was associated with 2 indicators at the local level: a positive strong association with SOD concentration and a negative strong association with MDA. On the other hand, the indicator of SOD local metabolic status was reliably associated with the three indicators of systemic metabolic status (Catalase, SOD, SCE). The other identified relationships were between single indicators (Table 6).

Table 1. Matrix of Spearman's multiple correlation between the indicators of metabolic and immune status in ischemic liver injury at the systemic level after correction with allogeneic hepatocytes

Indicators	MDA	AHP	TAA	SOD	Cat	SM_{ON}	Sum
AGC	-0,44	0,10	0,02	-0,25	***0,89***	0,28	1
MD	***0,61***	-0,3	-0,17	0,3	0,06	-0,18	1
KD	-0,07	0,28	-0,21	-0,3	-0,17	-0,5	-
PI	***-0,53***	0,29	-0,18	-0,39	0,25	0,18	1
PN	0,44	-0,19	-0,02	***0,78***	0,12	0,39	1
IPA	0,10	***-0,64***	-0,48	0,13	-0,26	-0,16	1
NBT -sp.	-0,28	0,37	0,16	-0,02	***0,6***	0,19	1
NBT- st. no/z	0,41	-0,2	-0,22	0,29	-0,01	-0,22	-
NBT-st. o/z	0,15	0,22	-0,08	0,26	***0,6***	-0,16	1
CA_{no}	0,11	-0,34	-0,13	0,26	***0,6***	0,08	1
CAo	0,09	0,06	0,52	***0,70***	0,3	0,37	1
CO	0,27	0,16	0,07	0,38	-0,19	0,26	-
Sum	2	1	-	2	4	-	9

Table 2. Matrix of Spearman's multiple correlation between the indicators of metabolic and immune status in acute liver ischemia after correction by allogeneic hepatocytes

Indicators	MDA	AHP	TAA	SOD	Catalase	SCE	SCG	Sum
AGC	***-0,6***	0,36	***0,9***	***0,6***	***0,6***	***0,81***	-0,05	5
MD	***0,7***	0,03	-0,32	-0,22	-0,15	-0,16	-0,12	1
KD	-0,26	-0,04	-0,06	-0,53	***-0,6***	0,09	0,5	1
PI	-0,44	0,07	0,42	-0,05	-0,08	0,18	0,23	-
PN	0,23	-0,23	-0,01	0,25	0,25	-0,02	-0,02	-
IPA	-0,11	0,07	0,001	0,07	0,05	-0,03	0,20	-
NBT - sp.	-0,18	-0,41	***0,72***	0,25	0,23	***0,86***	0,06	2
NBT - st. no/z	0,11	***-0,6***	0,11	-0,17	-0,17	0,39	0,4	1
NBT-st. o/z	-0,1	0,31	0,12	0,14	0,13	0,26	0,01	-
CAno	-0,1	0,2	0,45	0,23	0,25	0,40	-0,03	-
CAo	0,35	-0,24	0,08	0,5	0,49	0,14	***-0,63***	1
CO	0,45	-0,21	-0,45	-0,34	-0,34	***-0,6***	-0,05	1
Sum	2	1	2	1	2	3	1	12

Table 3. Matrix of Spearman's multiple correlation between the indicators of metabolic status in acute liver ischemia after correction with allogeneic hepatocytes

Indicators	Local level						
Systemic level	MDA	AHP	TAA	SOD	Cat	SM_{ON}	Sum
MDA	0,28	-0,14	-0,07	0,33	-0,21	0,04	-
AHP	-0,22	-0,13	-0,35	-0,43	0,32	-0,30	-
TAA	***-0,6***	0,12	0,13	-0,18	***0,81***	0,39	2
SOD	-0,38	-0,15	0,44	0,17	0,48	***0,6***	1
Catalase	-0,35	-0,2	0,44	0,15	0,48	***0,6***	1
SCE	-0,39	0,22	0,22	-0,01	***0,78***	0,15	1
SCG	0,42	-0,01	***-0,6***	-0,08	-0,20	-0,27	1
Sum	1	-	1	-	2	2	6

Table 4. Matrix of Spearman's multiple correlation between the indicators of metabolic and immune status in toxic liver damage at the systemic level after correction with allogeneic hepatocytes.

Показатели	MDA	AHP	TAA	SOD	Cat	SM_{ON}	Sum
AGC	0,02	0,2	0,13	-0,04	***-0,6***	0,1	1
MD	0,18	0,18	-0,18	-0,3	0,06	0,07	-
KD	***0,6***	0,37	0,03	***-0,6***	-0,07	-0,02	2
PI	0,06	0,07	0,01	0,02	-0,43	0,10	-
PN	-0,17	0,32	0,17	-0,3	0,02	0,17	-
IPA	0,33	-0,38	0,02	-0,1	-0,03	0,1	-
NBT - sp.	0,28	0,36	-0,25	-0,27	-0,32	-0,05	-
NBT - st. no/z	0,01	-0,1	0,07	-0,47	***-0,6***	0,2	1
NBT-st. o/z	0,30	-0,27	-0,1	-0,04	-0,41	0,03	-
CAno	0,01	-0,31	***0,70***	-0,1	0,12	***-0,82***	2
CAo	-0,03	0,05	-0,12	0,02	-0,44	0,3	-
CO	-0,32	-0,4	***-0,6***	-0,06	-0,03	-0,01	1
Sum	1	-	2	1	2	1	7

Table 5. Matrix of Spearman's multiple correlation between the indicators of metabolic and immune status in toxic liver damage at the local level after correction with allogeneic hepatocytes.

Indicators	Amount	Hb	MDA	AHP	TAA	SOD	Catalase	SCE	SCG	Sum
AGC	-0,02	0,33	***-0,79***	-0,16	0,03	***0,6***	0,06	-0,45	***0,6***	3

MD	-0,18	-0,34	0,35	-0,02	-0,07	0,39	-0,24	0,3	-0,19	-
KD	0,32	0,2	0,10	***0,6***	-0,2	0,25	***-0,81***	0,3	0,25	2
PI	0,22	0,01	-0,1	***0,6***	-0,25	-0,08	-0,28	-0,15	-0,09	1
PN	-0,25	0,06	0,43	***0,87***	-0,08	-0,13	-0,27	0,12	-0,27	1
IPA	0,44	0,18	***-0,62***	-0,21	0,17	0,01	-0,3	-0,25	***0,61***	2
NBT - sp.	-0,47	0,01	-0,005	-0,47	0,2	0,35	0,3	0,32	-0,2	-
NBT - st. no/z	-0,07	0,34	***-0,6***	0,15	0,03	0,27	-0,14	0,06	0,45	1
NBT-st. o/z	0,22	-0,31	-0,47	0,05	-0,03	-0,11	-0,14	-0,09	0,14	-
CAno	0,23	0,25	-0,13	-0,22	0,44	-0,07	0,01	0,37	-0,13	-
CAo	***-0,6***	-0,25	-0,09	-0,22	-0,12	0,03	0,45	0,09	-0,38	1
CO	0,12	***-0,6***	0,27	-0,34	0,24	0,10	0,19	0,13	0,06	1
Sum	1	1	3	3	-	1	1	-	2	12

Table 6. Matrix of Spearman's multiple correlation between the indicators of metabolic status at the local and systemic level in toxic liver damage after correction with allogeneic hepatocytes.

Indicators	**Local level**						
Systemic level	MDA	AHP	TAA	SOD	Cat	SM_{ON}	Sum
Hb	0,21	0,34	***0,64***	-0,17	0,053	-0,2	1
MDA	-0,33	0,06	-0,28	0,1	0,33	0,23	-
AHP	0,1	0,38	0,03	-0,28	0,1	0,34	-
TAA	0,06	-0,3	0,35	-0,2	-0,1	***-0,73***	1
SOD	0,22	0,43	0,05	***-0,6***	-0,36	-0,02	1
Catalase	***-0,68***	-0,34	-0,15	***0,6***	-0,17	-0,03	2
SCE	0,12	-0,05	0,04	***-0,64***	-0,33	-0,21	1
SCG	0,23	0,11	-0,07	-0,13	0,02	0,16	-
Sum	1	-	1	3	-	1	6

The last correlation analysis we carried out was similar to previous ones, however, we studied the relationships obtained in ethanol intoxication following the correction with allogeneic hepatocytes. When assessing the matrix of Spearman's multiple correlation between indicators of metabolic status at the local and systemic levels in ethanol intoxication, 6 significant correlations were identified. Thus, the dynamics of AHP and SM_{ON} level at the local level is associated with changes in 3 and 2, respectively, indicators of metabolic status at the systemic level, and the concentration of indicators at the systemic level - AHP and TAA - correlate with 2 indicators of the local level (Table 7).

When assessing the matrix of Spearman's multiple correlation between indicators of metabolic and immune status in alcoholic liver intoxication at the systemic level after correction with allogeneic hepatocytes, 13 significant correlations were revealed. Thus, the dynamics of the MDA level was associated with three indicators PN, NBT-sp. NBT-sp. no/z. The TAA and SM_{ON} dynamics correlates with two indicators NBT-sp. NBT-sp. no/ z. SOD reliably interacted with three indicators of immune status - AGC, PN, NBT-sp. no/z. Catalase correlated with AGC and NBT-cp. o/z. (Table 8). Having described the multiple correlations revealed between the indicators of metabolic and immune status in liver alcohol intoxication at the local level following the correction with allogeneic hepatocytes, we established 15 reliable direct and reverse relationships. AHP reliably corrected with 6 indicators (AGC, KD, PI, NBT-sp. NBT-sp. no/z). SOD corrected with two indicators (MD and PI). SM_{ON} was in a close correlation with IPA and CAn. SCE interacted with KD NBT-sp. SCG indicator correlated with the three indicators MD, NBT-sp. no/z, CO.

Table 7. Matrix of Spearman's multiple correlation between the indicators of metabolic status at the local and systemic level in ethanol intoxication after correction with allogeneic hepatocytes.

Indicators	**Local level**							
Systemic level	MDA	AHP	TAA	SOD	Catalase	SCE	SCG	Sum
MDA	0,13	***0,6***	0,07	-0,18	-0,21	-0,2	-0,4	1
AHP	-0,23	***-0,4***	***-0,6***	0,12	***-0,6***	-0,02	-0,1	2
TAA	0,04	***0,62***	-0,16	0,01	***-0,6***	-0,4	-0,4	2
SOD	0,11	***0,7***	-0,06	-0,22	-0,07	-0,41	-0,36	1
Catalase	-0,02	0,43	-0,24	-0,32	0,13	-0,31	-0,23	-
SM_{ON}	-0,21	-0,05	-0,28	-0,34	0,22	0,1	0,06	-
Sum	-	3	1	-	2	-	-	6

Table 8. Matrix of Spearman's multiple correlation between the indicators of metabolic and immune status in the liver ethanol intoxication at the systemic level after correction with allogeneic hepatocytes.

Indicators	**AGC**	**MD**	**KD**	**PI**	**PN**	**IPA**	**NBT – sp.**	**NBT – sp. no/z**	**NBT – sp. o/z**	**CAno**	**CAo**	**CO**	**Sum**
MDA	0,25	0,3	-0,2	-0,43	***0,62***	0,15	***0,71***	***0,73***	-0,3	0,03	-0,5	0,5	3
AHP	-0,3	-0,2	-0,1	-0,01	0,13	-0,23	-0,26	-0,4	-0,2	-0,4	-0,1	0,1	-
TAA	0,16	0,1	0,4	-0,4	0,45	-0,04	***0,63***	***0,85***	-0,3	-0,4	-0,1	0,1	2

SOD	***0,66***	0,2	0,32	-0,45	***0,64***	0,02	0,41	***0,79***	-0,5	-0,2	***-0,6***	0,2	4
Cat	***0,72***	0,1	0,18	-0,12	0,34	0,12	-0,1	0,23	***-0,6***	-0,2	-0,4	0,4	2
SM_{ON}	0,4	-0,1	-0,1	0,2	-0,2	-0,18	***-0,66***	***0,65***	-0,1	-0,3	-0,4	0,2	2
Sum	2	-	-	-	2	-	3	4	1	-	1	-	13

Table 9. Matrix of Spearman's multiple correlation between the indicators of metabolic and immune status in the liver ethanol intoxication at the local level after correction with allogeneic hepatocytes.

Indicators	**AGC**	**MD**	**KD**	**PI**	**PN**	**IPA**	**NBT – sp.**	**NBT – sp. no/z**	**NBT – sp. o/z**	**CAno**	**CAo**	**CO**	**Sum**
MDA	0,34	0,14	-0,3	0,2	0,3	0,32	-0,25	0,27	-0,03	0,2	-0,2	0,14	-
AHP	***0,62***	-0,2	***0,63***	***-0,7***	0,3	-0,16	***0,68***	***0,62***	***-0,65***	-0,1	-0,1	0,11	6
SOD	0,05	***0,68***	-0,4	***0,7***	-0,2	0,31	-0,16	0,1	0,43	0,3	-0,1	-0,3	2
Cat	-0,25	-0,4	-0,2	-0,2	-0,1	0,05	-0,02	0,13	-0,08	0,1	0,14	0,41	-
SM_{ON}	0,37	0,04	0,1	0,4	-0,2	***0,6***	-0,4	-0,4	-0,03	***0,6***	-0,1	0,04	2
SCE	0,08	-0,4	***-0,6***	0,1	-0,1	0,04	***-0,6***	-0,22	0,17	0,1	0,02	0,42	2
SCG	0,07	***-0,6***	-0,1	0,1	0,1	0,4	-0,4	***-0,6***	0,01	0,4	0,01	***0,6***	3
Sum	1	2	2	2		1	2	2	1	1	-	1	15

Specialized liver cells (hepatocytes) have become one of the first type of cells used for clinical purposes - cell therapy for patients with congenital and acquired liver defects. Interest in them from the scientific and practical point of view has now increased to an even greater extent due to the fact that the only way to treat liver failure, as the result of viral, autoimmune hepatitis, hereditary diseases and intoxication, is the lack of donor organs. Compared to precursor cells and stem cells, cultures of primary hepatocytes have a very limited ability to divide, which is a serious limiting factor for their practical use. The effectiveness of hepatocytes transplantation is significantly limited as a result of the development of immune homeostasis disorders [11].

Besides, the mechanism of hepatocytes action used to correct damaged liver tissue cannot be considered finally clarified. A number of authors believe that the therapeutic effect is associated with organ-replacing function. It has currently been proven that transplanted isolated stem cells, hepatocytes do not so much increase the functional mass of the liver, as change the humoral and molecular mechanisms responsible for the functional activation of the remaining hepatocytes of the recipient and regeneration through the production of low molecular weight humoral factors [12,13].

The obtained data allow us to conclude that the revealed activation of free-radical oxidation is a factor in the pathogenesis of many diseases. The data obtained in the research indicate pronounced changes in the immune reactivity and functional activity of polymorphonuclear leukocytes in presence of ischemic, toxic and alcohol liver damage, and the possibility to use allogeneic hepatocytes as well in the correction of the identified immune-metabolic disorders. It should be mentioned that the use of allogenic hepatocytes to a greater extent has a normalizing and corrective effect on the indicators of immune reactivity and functional activity of polymorphonuclear leukocytes.

The effects and action mechanisms of transplanted allogeneic hepatocytes, revealed during their introduction, are associated not so much with their organ-replacing function, as with the normalization and activation of liver autologous cells through humoral compounds (peptides, growth factors, cytokines, etc.), which change quantitatively and qualitatively the composition of circulating blood serum and through this content the ratio of proteins and lipids of the erythrocyte membrane [14-15].

What is more, the mechanisms of metabolic correction of implanted hepatocytes require further research. Isolation of the "effective" agent from the culture fluid of hepatocytes is rather challenging. One of the reason for this are the facts obtained earlier in our laboratory proving that not only the transplantation of allogeneic intact hepatocytes, but also the introduction of the culture fluid obtained on their basis, to recipients with experimental liver hypoxia, acute toxic hepatitis caused by carbon tetrachloride, significantly reduce the development of immune-inflammatory syndrome in the liver, normalize the synthetic function of hepatocytes, prevent the development of oxidative stress and impairment of innate immunity [16].

To bring the substitution cell therapy into more widespread use within clinical practice, further experimental studies are needed to determine the immune-metabolic effects of various options for regenerative cell therapy (use of xeno- and allogeneic transplants and their culture fluid), and their combined use with pharmacological preparations in liver pathology as well [17].

CONCLUSIONS.

1. Correlation analysis between the immune status and oxidative stress indicators proves the presence of interconnection and interdependence in the genesis of pathological changes occurring in the liver, which can help to assess the severity and effectiveness of the treatment.
2. The use of allogeneic hepatocytes has a pronounced positive effect on the parameters of the immune-metabolic status in acute ischemic, toxic and alcohol liver damage, which in its turn can be used in the treatment of these disorders.

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