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Research Article

Proteins of allogeneic hepatocytes and pharmacological preparations for the correction of immunometabolic disorders in experimental liver pathology

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Abstract

Introduction: The relationship of numerous metabolic shifts, disorders of hepatocytes functional activity resulting from hypoxia and toxic liver damage with the function of the immune system has not been sufficiently studied so far, nor have the most effective methods of pharmacological correction been established.

Materials and Methods: The studies were carried out on 603 mature male Wistar rats and 45 mice. Acute toxic liver damage (ATLD) was modeled by intramuscular introduction of carbon tetrachloride; acute liver ischemia (ALI) was caused by clamping the hepatoduodenal ligament for 20 minutes; chronic alcohol intoxication (CAI) was modeled by forced intragastric administration of 20% ethanol solution for 60 days. Isolation of xenogeneic (murine) and allogeneic (rat) hepatocytes from newborn mice and rats was carried out according to the method of Berry and Friend (1969); culture fluid of hepatocytes and its protein fractions were prepared according to our developed techniques. The obtained biological material was intraperitoneally introduced into the rats with ATLD, ALI, and CAI.

Results and Discussion: In all the models of the liver damage, there developed morphological and biochemical signs of the liver damage, impaired congenital and adaptive immunity, oxidative stress, increased lipid peroxidation processes.

Conclusion: The introduction of allogeneic hepatocytes, culture fluid obtained on their basis,and proteins isolated from it with MW less than 130 kDa to the recipients with toxic and ischemic liver damage more effectively corrects the pathologic changes in the liver in comparison with xenogeneic hepatocytes, their culture fluid and pharmacological preparations (combinations of Essentiale N and Hypoxenum or Heptral and Mexicor).

Keywords

correction of disorders by allogeneic hepatocytes and proteins of their culture fluid, toxic liver damage.

Introduction

The nature of any pathological process can be understood by exploring its biochemical, anatomical-morphological and dynamic functional connections with the clinical and physiological state of the body as a single whole. In this case, one of the most important aspects is to clarify the main structural and functional parameters of the affected tissue, organ or system that has a leading role in the disease pathogenesis (Anderson et al. 2018, Jabri et al. 2018).

The liver is responsible for the processing of metabolites entering the body, the synthesis of most blood plasma proteins, first of all albumins, fibrinogen, prothrombin, some globulin fractions, and many enzyme systems; it is directly involved in carbohydrate-lipid and vitamin metabolisms and digestion. The dynamics, sequence, qualitative and quantitative parameters, the limits of their changes in various pathological unitary, polyetiological, primary and secondary, acute and chronic, organ and systemic disorders are always of the greatest or decisive importance in explaining therapeutic, preventive and prognostic conclusions (Trubitsyna et al. 2016).

The problem of acute toxic liver damage (ATLD) treatment, complicated by the development of liver failure, remains a serious and urgent one. Damage to hepatocytes often occurs due to intake of hepatotoxic poisons, large doses of analgesic, anti-inflammatory, antibacterial, antimetabolic, and other drugs (Shikalova et al. 2012, Suyavaran 2017). Their entry into the body is characterized by a number of common signs – an increase in free radical processes and lipid peroxidation, a decrease in the activity of glucose-6-phosphatase, enzymes of NADPH-dependent monoxide system (especially, its main component, cytochrome P450), an increase in the permeability of cell membranes, fatty liver infiltration, and necrosis. The above mentioned changes underlie the development of cytolytic, cholestatic and hepatodepressive syndromes of liver damage. One of the most common effects caused by xenobiotics intake to the body is the labialization of lysosomal membranes. This significantly increases the "leakage" of hydrolytic enzymes of lysosomes into the blood plasma (Zabrodskiĭ et al. 2015, Konoplya et al. 2016).

Hypoxic conditions complicate the course of many diseases of various genesis, being the most important component of a wide variety of pathology nosological forms: all types of respiratory, cardiovascular failure, blood loss, myocardial ischemia, cerebral or peripheral circulation disorders, thermal and mechanical injuries. In surgical practice, it is often necessary to temporarily interrupt the blood supply to the organ being operated on, creating artificial ischemia. This determines the exceptional importance and social significance of protecting the body from oxygen deprivation and energy deficiency (Khodosovskii and Zinchuk 2006).

In clinical practice, for bloodless surgeries on the liver, the clamping of the hepatoduodenal ligament is often used. However, the resulting ischemia of the liver can be the cause of severe dysfunctions of this organ. An increase in the ligament clamping time by more than 15 minutes results not only in destructive processes in the liver, but also has a negative effect on the entire body as a whole (Liu et al. 2009, Reiling et al. 2015, Donadon et al. 2016). First of all, under conditions of liver ischemia, free radical processes and the resulting lipid peroxidation of cell membranes are enhanced. Accelerated formation of hydroperoxide radicals leads to the impairment of cell membranes and an increase in their permeability, as a result of which there is an increased release of cytoplasmic components, cellular organelles and products of disturbed cell metabolism into the vascular bed, which can directly change the functional activity of immunocompetent cells, as well as induce the emergence of immunosuppressive properties in erythrocytes, which is common in the pathogenesis of toxic hepatocytes damage (Khodosovskii and Zinchuk 2006, Warzecha et al. 2017).

Unsatisfactory results in the treatment of acute toxic and ischemic liver damages are largely associated with the lack of effective pathogenetic therapy, hence, a promising direction in the treatment of such conditions is the use of cellular technologies (Trubitsyna et al. 2016), and one of these areas is the use of isolated xeno- and allogeneic hepatocytes. Wherein, the effect from using such a transfer is believed to be associated not so much with organ-substituting function, but rather with humoral and molecular mechanisms responsible for the activation of the recipient's hepatocyte functions and regeneration through the production of regulatory peptides, among which the leading role belongs to growth factors (Danilova et al. 2020).

Hepatocytes have become the first cells type used for clinical purposes – cell therapy of patients with congenital and acquired liver pathology (Ostroverkhov et al. 1979, Bruslik et al. 1994). In comparison with progenitor cells and stem cells, hepatocyte cultures have a very limited ability to divide, which is a serious limiting factor for their practical use, but under stressful conditions (including acute liver damage), they fall into hyperplasia and acquire the ability to actively reproduce. The given property of hepatocytes has provided the basis for their use in the processes of liver diseases recovery (Gandillet et al. 2005, Jabri et al. 2018, Evseeva et al. 2021).

Problem

To enhance our insight about the role of the liver in the diseases pathogenesis, to develop qualitative and quantitative assessment criteria of its morphological and functional indicators in dynamics is a difficult, multifaceted, but extremely urgent task for current knowledge (Chikoteev et al. 2003, Konoplya et al. 2016).

The frequency of acute and chronic liver diseases in the general structure of human diseases and mortality from this type of pathology are steadily increasing even in economically developed countries. The problems of pathogenesis, diagnostics and treatment of acute and chronic liver diseases remain among the most urgent in medicine, both due to the complicacy of their diagnosis and a choice of optimal effective treatment methods, and due to an upward trend in the number of patients with these diseases (Shikalova et al. 2012, Got'e et al. 2013).

The relationship of numerous metabolic shifts, disorders in the functional activity of hepatocytes arising from hypoxia and toxic liver damage with the immune system function has not been sufficiently studied so far, nor have

the most effective methods of pharmacological correction been established (Trubitsyna et al. 2016).

Despite modern medicine achievements, pharmacotherapy of acute and, especially, chronic liver diseases is an urgent problem, since the use of standard treatment regimens is often ineffective with a high mortality rate in this category of patients. The organ transplantation is still the main treatment choice for severe liver diseases. Due to the lack of donor material and post-operation problems, methods of cell replacement therapy for liver diseases are being developed. An essential experimental and clinical material which have been collected over the last few years shows that cell therapy can be considered as one of the priority directions in modern biomedicine and biotechnology (Shalakhmetova et al. 2009, Danilova et al. 2020).

At present, it can be argued that oxidative, immune disorders and changes in the structural and functional properties of erythrocytes – a kind of "cellular dosimeter" of the action of pathogenic factors – have an important role in damage and regeneration of liver cells by various etiological factors (Gandillet et al. 2005, Liu et al. 2009, Minasyan 2014, He et al. 2017, Pretini et al. 2019). There are a large number of experimental and clinical papers on the correction of liver dysfunctions, including the use of cellular technologies (Trubitsyna et al. 2016, Danilova et al. 2020); there are studies on oxidative, immune and erythrocytic disorders and their correction in liver pathology (Konoplja et al. 2017), but in fact there are no papers concerning the corrective effect of xeno- and allogeneic cells and transplantation of their cultural humoral factors.

The lack of effective means and methods to correct the disorders of the immune and metabolic statuses and to prevent morphological changes in the liver tissue due to its damage determines the relevance to use the culture fluid of allogeneic hepatocytes initially under experimental conditions, both separately and in combination with pharmacological preparations (antioxidants and membrane protective agents).

The aim of the study

The aim of the study: to determine the effectiveness of humoral factors of the protein-based nature in xenogeneic and allogeneic hepatocytes in the correction of metabolic, immune and morphological disorders in experimental liver pathologies of toxic and ischemic etiology.

Materials and methods

Animals

The experiments were carried out on 603 sexually mature male Wistar rats, weighing 160–200 g; 65 Wistar rats and 45 mice were taken into the experiment on the 5–6th days after birth as donors of hepatocytes. All the studies were carried out at the same time of the day, from 8 am to 12 pm, in compliance with the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, France, 1986), and in accordance with the rules of laboratory practice of the Russian Federation (Order No. 267 of the Ministry of Health of the Russian Federation, dated June 19, 2003).

Design of the Experiment

Acute toxic liver damage (ATLD) was simulated by intraperitoneal introduction of 3 ml/kg of carbon tetrachloride (CTC) in 50% olive-oil solution, five times, at a 24-hour interval (Smakhtin et al. 2002).

Acute liver ischemia (ALI) was induced under intraperitoneal Hexenal anesthesia by clamping the hepatoduodenal ligament for 20 minutes.

Chronic alcohol intoxication was modeled by forced intragastric administration of 20% ethanol solution at a dose of 3 ml/kg (2.92 g/kg) every 24 hours for 60 days (Dolgareva et al. 2018).

Preparation of xenogeneic and allogeneic hepatocytes

Isolation of xenogeneic (murine) and allogeneic hepatocytes (XH, AH) was carried out according to the method of Berry and Friend (1969) from 5–6-day-old animals. After the liver was withdrawn, it was reduced to small pieces in medium 199, and the liver cells were extracted from the tissue by extrusion, using a glass homogenizer. The obtained cell suspension was washed twice by centrifugation for 10 min at 400 g and, after the supernatant was removed, it was diluted in medium 199, then the number of cells was counted in Gorjaev's chamber. Cell viability was determined in a stain test – with trypan blue, while cell suspensions containing less than 90% of viable cells were not used. Following the concentration by centrifugation, a pool of cell suspension from 2–3 rats or 3–4 mice at a concentration of 2×10^6/kg was immediately intraperitoneally introduced, ten times, with 24 hours apart, in the volume of 0.5 ml in medium 199, starting from the 51st day of ethanol intoxication, within 5 days to the rats with ALI or five times simultaneously with CTC in the dynamics of ATLD development. Hepatocytes were prepared daily and immediately introduced after preparation. During all the procedures with the cell suspension, the temperature of the used medium 199 was 36–37 °C (Konoplya 1985, Konoplya et al. 2016).

Preparation of the culture fluid of xenogeneic and allogeneic hepatocytes

In order to obtain the culture fluid of xenogeneic and allogeneic hepatocytes (CFXH, CFAH), 5×10^7 cells per 3 ml of medium were cultured in medium 199 for 6 hours. Following the expiration of incubation period, the cells (for 15 min at 400 g). Protein concentration in the culture fluid was determined using Coomassie Brilliant Blue G-250. Cultural fluids from hepatocytes were prepared daily and introduced into rats immediately after modeling liver ische-

mia or simultaneously with CTC, intraperitoneally five times, with 24 hours apart, or ten times, starting from the 51st day of ethanol intoxication, at 5 mg/kg of body weight.

To obtain hepatocytic proteins from 50–100 ml of culture fluid, they were precipitated in equal volumes with 10% triacetic acid for 30 minutes; the precipitate was separated by centrifugation for 30 minutes at 3000 g. After the supernatant was separated, the sediment was diluted with alkalized solution of 0.9% sodium chloride and dialyzed in phosphate-buffered saline with pH 7.2–7.4 for 18 hours (Konoplya 1985). The dialyzed protein solution was clarified by centrifugation at 3000 g for 30 minutes and fractionated on a ULTROGEL AcA44 column. Protein from the column came out in two peaks: the first one in the free volume contained proteins of more than 130 kDa; the second one, the main peak, which was entrapped in this gel, contained proteins less than 130 kDa. Having determined the protein concentration in the peaks, the obtained fractions were filtered through 0.2-μm sterilizing membranes, dispensed into sterile vials, with 1 mg of protein per vial, and lyophilized on a VIRTIS freeze dryer. The obtained proteins of allogeneic hepatocytes (PAH) with MW of more and less than 130 kDa were introduced five times (with a 24-hour interval) intraperitoneally at the rate of 5 mg/kg of protein (Jabri et al. 2018, Litvinova et al. 2019) from the 55th day of intoxication with ethanol, CTC, or immediately after ALI modeling.

Poisoning with hepatotropic poison, alcohol intoxication in the doses and frequency of administration used, or modeling of acute liver ischemia, according to the literature and in our experiments did not result in the animals' death during the experiment; the animals were withdrawn from the experiment 24 hours after the last introduction of CTC, alcohol, hepatocytes, their culture fluid, the proteins of culture fluid, or pharmacological preparations.

The study design and the number of animals used are presented in Table 1.

The dosage, methods and administration frequency of the pharmacological preparations are presented in Table 2. Calculation of preparation dosages for administration to the experimental animals was carried out by means of dose conversion factor (mg/kg per mg/m^2) for rats depending on body weight (Freireich 1966) or empirically, based on LD_{50}.

Blood sampling from the experimental animals was carried out under anesthesia by intracardiac puncture. Plasma and erythrocytes were obtained from heparinized blood by centrifugation for 5 min at 400 g. To assess the functional state of hepatocytes in the blood plasma, we determined the thymol test (TT), the activity of aspartate- and alanine aminotransferases (AST, ALT), alkaline phosphatase (AP), gamma glutamyl transpeptidase (GGT), the contents of bilirubin (BR), fibrinogen (FG), and prothrombin index (PTI). The values of all the above listed parameters were determined by unified methods using standard reagent kits. Liver enzymes activity was assessed on an automatic biochemical analyzer Vitalab Flexor E (Netherlands), with Analyticon Biotechnologies AG reagents (Germany). The fibrinogen content was determined by means of Diagnostica Stago STart 4 Hemostasis Analyzer (France).

The intensity of lipid peroxidation (LPO) processes was assessed by the contents of acyl hydroperoxides (AHP) and malondialdehyde (MDA) in plasma and erythrocytes, by means of a TBK-Agat kit (Agat-Med, Russia) when using an Apel-330 spectrophotometer (Japan) at wavelengths of 535 nm and 570 nm. By the method of direct/competitive enzyme-linked immunosorbent assay (ELISA) with the detection of reaction products at wavelengths ranging from 405 to 630 nm; the condition of antioxidant system was assessed using the kits to measure the activity of superoxide dismutase (SOD) manufactured by Bender Medsystems (Austria) and to measure the activity of catalase manufactured by Cayman Chemical (USA). The total antioxidative activity (TAA) was determined by a method based on a degree of ascorbate inhibition and ferro-induced oxidation of Tween-80 to MDA. The level of stable nitric oxide metabolites (SM_{ON}) was determined using an ELISA kit from R&D (England). All the ELISA results were recorded using a Sunrise microplate photometer (Tecan, Austria).

Isolation of neutrophils from the blood obtained was carried out, using the Ficoll-Urografin density gradient method (ρ = 1.078). Their phagocytic activity was assessed by the phagocytic index (PI), phagocytic number (PN), and the index of phagocyte activation (IPA). The activity of oxygen-dependent systems of neutrophils was assessed by the reduction reaction of nitro-blue tetrazolium (NBT-test), spontaneous and stimulated by zymosan (NBT-sp, NBT-st, coefficients of opsonization and activation to opsonized and non-opsonized zymosan (CO, CA_O, CAn) (Zinkin and Godkov 2004).

The development of a humoral immune response (HIR) to sheep erythrocytes (SE) was assessed on the 5th day following the immunization, based on the content of antibody-producing cells (APC) in the spleen. Delayed hypersensitivity (DHS) was induced by intraperitoneal introduction of SE (a sensitizing dose). Four days later, a challenge dose of SE was injected into the right footpad. The intensity of DHS was assessed by the difference in the masses (DM) of regional and contralateral lymph nodes and by the difference in karyocyte number (DK) in them (Fedoseeva et al. 1993).

A histological examination of the liver was carried out to morphologically confirm the development of simulated pathological changes and to comprehensively assess the effectiveness of correcting the disorders. Pieces of the organ were fixed in 10% neutral formaline in 0.1 M phosphate buffer (pH = 7.2) and embedded in paraffin. Paraffin sections, 7–10 μm thick, were stained with hematoxylin and eosin. A microscopic examination of histological preparations was carried out using a Nikon Eclipse Ci-S biomedical microscope with a standard digital camera manufactured by Nikon Corporation, Japan, at different objective magnifications (×4, ×10, ×20, ×40).

Table 1. The Study Design and Grouping of Animals

Series no.	Experimental conditions	Group No	Experimental conditions in a group	Number of animals
1	Morphofunctional disorders in acute toxic liver damage; corrective activity of xeno-, allogeneic hepatocytes, their culture fluid without and with pharmacological preparations	1	Intact	12
		2	Introduction of olive oil and 0.9% sodium chloride solution	11
		3	Introduction of Essentiale N and Hypoxenum	11
		4	Administration of Heptral and Mexicor	12
		5	Introduction of xenogeneic hepatocytes	12
		6	Introduction of allogeneic hepatocytes	11
		7	Introduction of culture fluid of xenogeneic hepatocytes	12
		8	Introduction of culture fluid of allogeneic hepatocytes	12
		9	Introduction of culture fluid of allogeneic hepatocytes, Essentiale N and Hypoxenum	12
		10	Introduction of culture fluid of allogeneic hepatocytes, Heptral and Mexicor	12
2	Correction of immunometabolic disorders by xeno- and allogeneic hepatocytes and their culture fluid without and with pharmacological preparations in acute toxic liver damage	1	Intact	10
		2	Introduction of olive oil and 0.9% sodium chloride solution	10
		3	Introduction of Essentiale N and Hypoxenum	10
		4	Administration of Heptral and Mexicor	12
		5	Introduction of xenogenic hepatocytes	12
		6	Introduction of allogeneic hepatocytes	11
		7	Introduction of culture fluid of xenogenic hepatocytes	11
		8	Introduction of culture fluid of allogeneic hepatocytes	10
		9	Introduction of culture fluid of allogeneic hepatocytes, Essentiale N and Hypoxenum	11
		10	Introduction of culture fluid of allogeneic hepatocytes, Heptral and Mexicor	12
3	Acute liver ischemia: morphofunctional disorders in the organ, their correction by xeno- and allogeneic hepatocytes and their culture fluid without and with pharmacological preparations	1	Intact	12
		2	Introduction of olive oil and 0.9% sodium chloride solution	11
		3	Introduction of culture fluid of xenogenic hepatocytes	11
		4	Introduction of Essentiale N and Hypoxenum	12
		5	Administration of Heptral and Mexicor	12
		6	Introduction of culture fluid of allogeneic hepatocytes	11
		7	Introduction of culture fluid of allogeneic hepatocytes, Essentiale N and Hypoxenum	12
		8	Introduction of culture fluid of allogeneic hepatocytes, Heptral and Mexicor	12
4	Immunometabolic disorders and their correction by xeno-, allogeneic hepatocytes, their culture fluid without and with pharmacological preparations in acute liver ischemia	1	Intact	12
		2	Introduction of olive oil and 0.9% sodium chloride solution	11
		3	Introduction of culture fluid of xenogenic hepatocytes	11
		4	Introduction of Essentiale N and Hypoxenum	12
		5	Administration of Heptral and Mexicor	12
		6	Introduction of culture fluid of allogeneic hepatocytes	11
		7	Introduction of culture fluid of allogeneic hepatocytes, Essentiale N and Hypoxenum	12
		8	Introduction of culture fluid of allogeneic hepatocytes, Heptral and Mexicor	12
5	The use of xeno- and allogenic hepatocytes and their culture fluid for the correction of metabolic disorders in presence of chronic alcohol intoxication	1	Intact	12
		2	Introduction of olive oil and 0.9% sodium chloride solution	11
		3	Introduction of xenogenic hepatocytes	11
		4	Introduction of allogeneic hepatocytes	11
		5	Introduction of culture fluid of allogeneic hepatocytes	12
6	The effectiveness of proteins of culture fluid of allogenic hepatocytes in the correction of immunometabolic disorders in acute toxic liver damage	1	Intact	12
		2	Introduction of olive oil and 0.9% sodium chloride solution	11
		3	Introduction of culture fluid of allogeneic hepatocytes	10
		4	Introduction of proteins of culture fluid of allogeneic hepatocytes	11
		5	Introduction of proteins of culture fluid of allogeneic hepatocytes with MW less than 130 kDa	12
		6	Introduction of proteins of culture fluid of allogeneic hepatocytes with MW more than 130 kDa	11
7	The effectiveness of proteins of culture fluid of allogenic hepatocytes in the correction of immunometabolic disorders in acute ischemic liver damage	1	Intact	12
		2	Introduction of olive oil and 0.9% sodium chloride solution	11
		3	Introduction of culture fluid of allogeneic hepatocytes	10
		4	Introduction of proteins of culture fluid of allogeneic hepatocytes	11
		5	Introduction of proteins of culture fluid of allogeneic hepatocytes with MW less than 130 kDa	12
		6	Introduction of proteins of the culture fluid of allogeneic hepatocytes with MW more than 130 kDa	11
8	Comparative laboratory corrective efficacy of immunometabolic disorders of xeno- and allogeneic hepatocytes, their culture fluid, proteins of culture fluid without and with pharmacological preparations in acute liver pathology			
Total				603

Table 2. Dosages, Methods and Frequency of Preparation Administration to Animals with an Experimental Model

Preparation	Way of introduction	Single dose, mg/kg	Injection scheme	
			Number of injections (introductions)	Interval between injections (introductions), h
Essentiale N	intragastrically	5 mg, calculated as phosphatidylcholine, dissolved in 1 ml of olive oil	30	24
Hypoxenum	intragastrically in 1% starch suspension	750	14	24
Mexicor	intraperitoneally	50	14	24
Heptral	intraperitoneally	760	5	24

Statistical processing of the results

To analysis and compare the qualitative indicators, we used the χ^2 criterion, and for the quantitative indicators – Student's t-test criterion after assessing whether the quantitative features were within the normal distribution (Shapiro-Wilk's test.). The differences were considered significant at $p < 0.05$.

A degree of disruption of the laboratory parameters (1) and a degree of changes of the laboratory parameters (2) under the influence of the pharmacological agents were calculated using special formulas (Zemskov et al. 2005, Uemoto et al. 2016).

$$\left[\frac{\text{Index of a certain animal}}{\text{Index of the norm}} - 1\right] \times 100\% \qquad (1)$$

$$\left(1 - \frac{\text{of animals with the } 2^{nd} - 3^{d} \text{ degrees of index disruption following the introduction by the methods developed}}{\text{of animals with the } 2^{nd} - 3^{rd} \text{ degrees of index disruption without introduction of the preparations}}\right) \times 100\% \qquad (2)$$

Results and discussion

Acute toxic liver damage

When introducing Heptral, Mexicor, Essentiale N, Hypoxenum or their combinations to the intact animals, there were no significant differences between the studied parameters of the immune response, functional activity of peripheral blood neutrophils, blood plasma parameters of hepatocyte functional activity, LPO markers, or factors of antioxidant defense.

In the animals with five-fold CTC poisoning, the development of main biochemical syndromes of liver damage was revealed, such as: cytolysis (an increase in the activity of AST, ALT, a decrease in de Ritis ratio), intrahepatic and extrahepatic cholestasis (an increase in the activity of AP and GGT), intracellular cholestasis with jaundice and toxic damage to hepatocytes (an increase in the content of BR, the activity of ALT, AST, AP, GGT, the values of de Ritis and GGT/AST ratios less than 1), insufficiency of synthetic processes (a decrease in PTI and FG) and immunoinflammatory (an increase in TT) (Table 3).

Simultaneous introduction of Essentiale N and Hypoxenum with CTC poisoning normalizes PTI and corrects the rest of the biochemical parameters characterizing the hepatocyte functional activity, with the exception of GGT/AST enzyme coefficient, to those of the healthy animals, but never reaches their level, The use of Heptral and Mexicor, in comparison with Essentiale N and Hypoxenum, corrects the ALT activity and the GGT/AST ratio to a greater extent, but even more reduces de Ritis ratio.

Simultaneous introduction of XH with CTC, in comparison with intoxication, corrects the activity of all the studied enzymes, de Ritis ratio, PTI, TT, BR and the FG level towards the indicators of the healthy animals, and reduces GGT/AST coefficient towards the healthy donors. The AH use turned out to be more effective, because their introduction, in comparison with XH, corrects the activities of AST, ALT, GGT, the PC concentration, PTI, and TT to a greater extent.

The use of CFXH normalizes the activity of AP and GGT and corrects the activities of AST, BR, the FG concentration, and the AST/ALT coefficient and TT towards the indicators of the healthy animals. The use of CFAH turned out to be the most effective, since its administration normalized, in addition to the effect from CFXH, the AST activity, the GGT/AST coefficient and corrected the ALT activity, de Ritis ratio and FG level towards the norm (Table 3).

In joint use of the pharmacological preparations and CFAH, it was found out that there were no special differences in the studied biochemical parameters characterizing the hepatocyte functional activity in presence of ATLD, except for the normalization of BR, the FG content and an additional TT correction, with the joint administration of CFAH, Essentiale N and Hypoxenum, where-

Table 3. The Impact of Pharmacological Preparations and CFAH Joint Action on the Functional Activity of Hepatocytes in Acute Toxic Liver Damage (M±m)

Indices	Unit of measurement	1	2	3	4	5
		Control	CTC poisoning and introduction of:			
			Olive oil and culture medium	CFAH	CFAH, Essentiale N and Hypoxenum	CFAH, Heptral and Mexicor
AST	U/L	29.2±2.4	55.1±4.2^{*1}	33.3±2.7^{*2}	31.3±3.1^{*2}	29.9±4.2^{*2}
ALT	U/L	22.4±1.9	89.5±5.1^{*1}	30.8±3.3^{*1,2}	29.1±2.8^{*1,2}	31.3±2.7^{*1,2}
AP	U/L	231.5±17.9	461.8±34.2^{*1}	263.3±12.1^{*2}	251.4±14.5^{*2}	243.7±13.0^{*2}
de Ritis ratio, AST/ALT		1.3±0.03	0.62±0.05^{*1}	1.1±0.05^{*1,2}	1.1±0.04^{*1,2}	0.96±0.03^{*1-4}
GGT	U/L	5.9±0.2	19.6±2.2^{*1}	5.6±0.4^{*2}	5.8±0.3^{*2}	6.1±0.2^{*2}
GGT/AST		0.2±0.01	0.36±0.02^{*1}	0.17±0.02^{*2}	0.19±0.02^{*2}	0.2±0.02^{*2}
BR	μmol/L	5.4±0.3	18.5±1.2^{*1}	6.3±0.3^{*1,2}	5.8±0.3^{*2,3}	6.1±0.2^{*1,2,4}
PTI	%	62.3±3.8	46.8±3.5^{*1}	56.1±3.1^{*2}	60.1±3.7^{*2}	61.4±4.1^{*2}
FG	g/L	4.1±0.1	2.3±0.03^{*1}	3.8±0.1^{*1,2}	3.9±0.1^{*2}	3.8±0.05^{*1,2}
TT	U. S-H	2.5±0.04	4.3±0.1^{*1}	3.0±0.05^{*1,2}	2.8±0.1^{*1-3}	2.9±0.06^{*1,2}

Note: Here and in the tables to follow, an asterisk marks significant differences in arithmetic means ($p < 0.05$); the numbers next to the asterisk – in relation to the indicators of what group these differences are given.

as there was a more pronounced shift of de Ritis ratio towards that of the control animals with the simultaneous use of CFAH with Heptral and Mexicor in comparison with the introduction of the culture liquid of allogeneic hepatocytes only (Table 3).

The introduction of proteins of CFAH with MW of more than 130 kDa did not affect the biochemical parameters of hepatocytes, which had been altered due to hepatotropic poisoning. The use of CFAH normalized PTI, the activity of AST, AP, GGT and the GGT/AST ratio and brought the values of PTI, TT, ALT activity, BR and FG levels closer to the values of the control animals, but not to their level. The introduction of whole (not divided into fractions) proteins of CFAH has almost the same effect, with the exception of additional normalization of the ALT activity and the FG concentration and the correction of TT. Proteins of CFAH with MW less than 130 kDa, in comparison with undivided proteins of CFAH, normalize the BR content.

When studying the metabolic parameters of blood plasma in ATLD: the LPO state, the antioxidant defense factors and the level of constant nitric oxide metabolites, it was found that in the poisoned animals, LPO processes were increased (the concentrations of MDA and AHP were increased), the antioxidant defense indicators (TAA, SOD and catalase activity) and the SM_{ON} concentration were reduced (Fig. 1).

The poisoning of the experimental animals with CTC causes the development of LPO and oxidative stress (an increase in MDA and AHP, a decrease in TAA, SOD, catalase activity) at the local level (erythrocytes) (Fig. 2).

The use of Essentiale N and Hypoxenum in presence of ATLD brings the LPO parameters, the catalase activity and the SM_{ON} concentration in blood plasma, and the most of the studied metabolic parameters closer to the parameters of the healthy animals, but not to their level. The introduction of Heptral and Mexicor, in comparison with Essentiale N and Hypoxenum, additionally normalizes TAA and corrects the SOD activity and the SM_{ON} content in blood plasma. In erythrocytes, the concentration of MDA and the activity of catalase are normalized.

The introduction of XH corrects the metabolic parameters of blood plasma (with the exception of the MDA concentration), normalizes TAA in erythrocytes and corrects the activity of antioxidant enzymes (SOD and catalase). The use of AHs turned out to be more effective, since their administration, in comparison with XHs, additionally normalized the activity of TAA and catalase in blood plasma, and, in erythrocytes, to a greater extent, brought the concentration of AHP and SOD activity closer to the SCG (sorption capacity of glycocalyx) control values.

The introduction of CFXH corrects the concentrations of LPO and SM_{ON} products in blood plasma, normalizes the catalase activity, and in erythrocytes normalizes TAA and to a greater extent brings the levels of MDA, AHP and SOD activity to the normal values. The use of CFAH had a more pronounced effect in comparison with CFXH, because it additionally normalized the SOD activity, corrected the contents of LPO and SM_{ON} products in blood plasma, and normalized the catalase activity in erythrocytes and brought the SOD activity closer to the norm (Figs 1, 2).

The combined administration of CFAH and the pharmacological preparations (Essentiale N and Hypoxenum or Heptral and Mexicor), in comparison with CFAH, corrected to a greater extent the contents of MDA, stable nitric oxide metabolites in blood plasma.

The introduction of CFAH proteins with MW more than 130 kDa did not affect the development of oxidative stress at either the general (blood plasma) or local (erythrocytes) levels and the sorption parameters of red blood cells caused by hepatotropic poison. The introduction of CFAH normalizes the parameters of antioxidant defense (TAA, SOD, catalase in blood plasma, TAA and catalase in erythrocytes) and corrects the rest of the studied biochemical parameters at the systemic and local levels. The use of either undivided CFAH proteins or CFAH proteins with MW less than 130 kDa in addition to the previous groups normalizes the SOD activity in erythrocytes.

ATLD increases the formation of humoral immune response (HIR) and cellular (DHS) immune response induced by SE, as evidenced by a significant increase in immune APC, DM and DK in the poisoned animals. The introduction of Essentiale N with Hypoxenum, Heptral with Mexicor corrects the indicators of HIR and DHS development towards the parameters of the healthy donors, but not to their level. The use of XH has a similar effect on HIR and to an even greater extent corrects the formation of DHS to SE. The introduction of AH, similarly to XH, affects DHS, but to a greater extent brings the APC count in the spleen closer to those in the control group.

There was an increase in the majority of indicators of phagocytic and oxygen-dependent activities in peripheral blood neutrophils, with an exception of neutrophilsfunctional backups CAn (norm), CAo (decrease) and the indicator of a response intermittency degree to opsonized and non-opsonized zymosan – CO (decrease).

In the group of the animals with ATLD treated with Essentiale N and Hypoxenum, the parameters of phagocytic activity (PI and IPA) shifted towards the healthy donors, but never reached their levels, and among the indicators of the oxygen-dependent neutrophil activity, the intermittency value (CO) was normalized and one of the parameters of polymorphonuclear leukocyte functional backup (CAo) was corrected. In contrast to the previous group, the use of Heptral and Mexicor additionally changed four parameters of neutrophil FMA (correction of PN, NST-sp, an increase in CAn and CAo).

When introducing XH, a correction of all the indicators of circulating neutrophil phagocytic activity (PI, FN, IPA) was observed, and among the parameters of oxygen-dependent activity there was the normalization of CA_n, CO, correction of NBT-st. n/z and a CAo increase. In comparison with XH, AH transplantation to the recipients with ATLD brought the phagocytic activity of polymorphonuclear blood leukocytes (FN and IPA) closer to the control parameters.

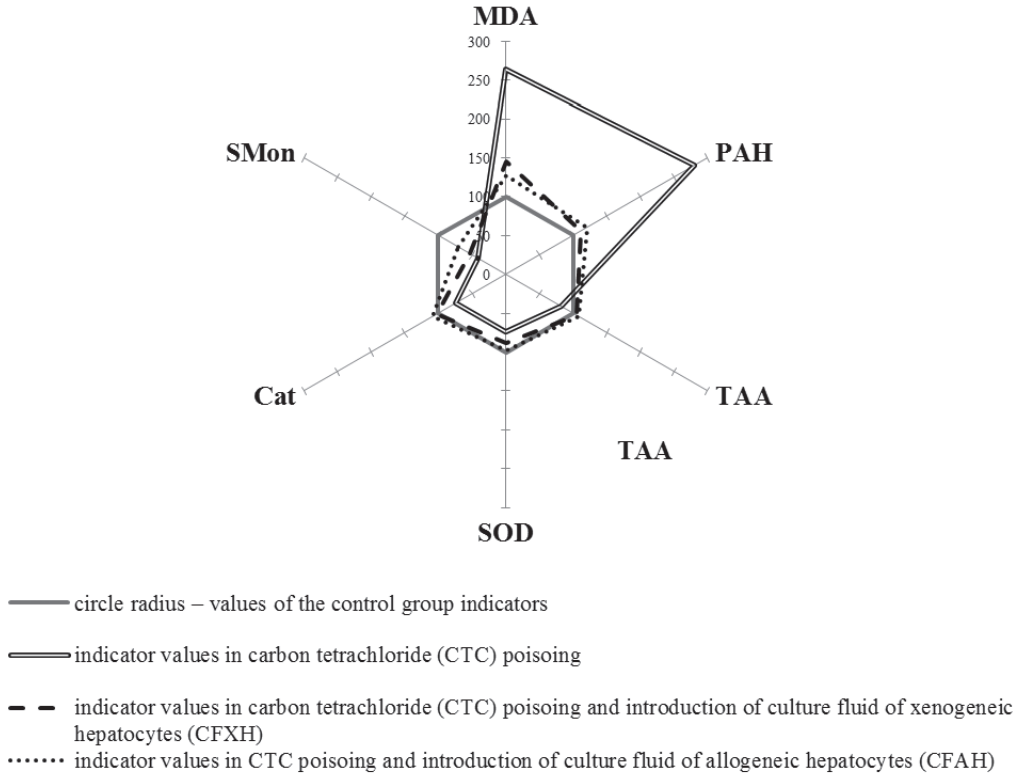

Figure 1. Influence of culture fluid of xeno- and allogeneic hepatocytes on metabolic parameters of the blood plasma. **Note:** DA – malondialdehyde; PAH – proteins of allogeneic hepatocytes; TAA – total antioxidative activity; SOD – superoxide dismutase; Cat – catalase; CM_{ON} – constant nitric oxide metabolites.

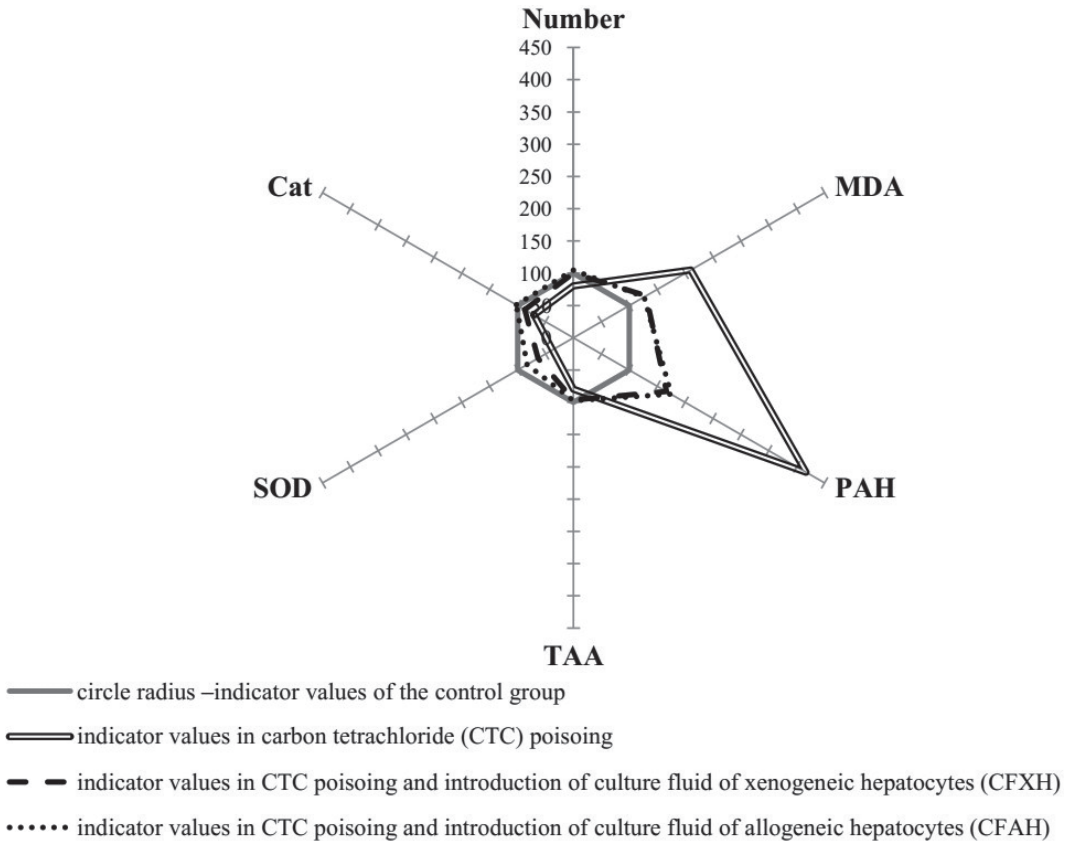

Figure 2. Influence of culture fluid of xeno- and allogeneic hepatocytes on metabolic parameters of erythrocytes. **Note:** MDA – malondialdehyde; PAH – proteins of allogeneic hepatocytes; TAA – total antioxidative activity; SOD – superoxide dismutase; Cat – catalase.

The introduction of CFXH was found to correct the development of HIR and DHS, induced by SE (APC, DM and DK). The use of CFAH, in comparison with the previous groups of the experimental animals, is more effective for HIR formation as evidenced by a greater decrease, but not to the control parameters, in immune APCs in the spleen of rats with ATLD.

The introduction of CFXH to a greater extent brings the phagocytic activity of polymorphonuclear leukocytes and NBT-st n/z and o/z to the values of the healthy animals, but not to their level, normalizes NBT-sp, and increases the functional backup of neutrophils. CFAH has the highest efficiency since its administration normalized 7 and more efficiently corrected 3 out of 10 investigated parameters of neutrophil FMA.

The use of CFAH proteins with MW more than 130 kDa in these conditions did not affect the development of adaptive and innate immunity, which was evidenced by the absence of changes in the corresponding parameters, in comparison with ATLD. The introduction of CFAH normalized the phagocytic activity of polymorphonuclear leukocytes in the circulating blood and two indicators of metabolic activity (NBT-st. n/z and CO) and brought other investigated indicators of immunity closer to the values of control ones. The introduction of whole CFAH proteins additionally normalized oxygen-dependent activity of neutrophils, while CFAH proteins with MW less than 130 kDa normalized only one DHS indicator – DK.

Thus, in acute CTC intoxication, the development of the main biochemical syndromes of liver damage (cytolysis, intrahepatic and extrahepatic cholestasis, intracellular cholestasis with jaundice and toxic damage to hepatocytes, insufficient synthetic and immunoinflammatory processes) was registered. The introduction of culture fluid of hepatocytes from the intact newborn rats to the allogeneic recipients in presence of CTC intoxication, in comparison with the culture fluid of xenogeneic hepatocytes, two combinations of a hepatoprotector with an antioxidant, more effectively corrects the changes in biochemical parameters of hepatocyte functional activity arising from hepatotropic poisoning.

The joint administration of the pharmacological preparations (Essentiale forte N and Hypoxenum or Heptral and Mexicor) with the culture fluid of allogenic hepatocytes in presence of CTC intoxication does not change its effectiveness.

The highest hepatoprotective, immunomodulatory, and antioxidant activities in ATLD are manifested in CFAH proteins with MW less than 130 kDa.

An analysis of morphological changes in the liver tissue in the animals with acute toxic damage showed that the correction of disorders caused by exposure to carbon tetrachloride was most effective when administering CFAH proteins with MW less than 130 kDa.

Acute liver ischemia

Having modeled ALI, we revealed the changes that indicate the development of the main biochemical syndromes of liver damage in the animals: cytolysis, intrahepatic and extrahepatic cholestasis, intracellular cholestasis with jaundice and toxic damage to hepatocytes, insufficient synthetic and inflammatory processes.

The introduction of Essentiale N with Hypoxenum or Heptral with Mexicor combinations almost equally neutralizes the parameters of hepatocytes functional activity in ALI: they normalize PTI, correct most of the studied biochemical parameters towards the values of the healthy animals, but not to the normal level, with the exception of the GGT/AST ratio (it does not change) and the the AST/ALT ratio (it is corrected to a greater extent due to the introduction of Heptral and Mexicor). The use of XH, in comparison with Heptral and Mexicor. to a greater extent reduces the GGT activity, the GGT/AST ratio, TT and increases de Ritis ratio. The use of AH as opposed to XH was somewhat more effective, since TT and the GGT activity decreased to a greater extent, but not to the control level.

The use of CFXH in the experimental animals with acute liver hypoxia does not affect the GGT/AST ratio, but normalizes PTI, the enzymatic coefficients and brings TT, the activity of ALT, AP and the fibrinogen level closer to the values of the healthy animals. The introduction of CFAH normalizes PTI, the AST/ALT and GGT/AST ratios and corrects the other investigated parameters of hepatocytes functional and metabolic activity in ALI. The joint use of the pharmacological preparations Essentiale N and Hypoxenum or Heptral and Mexicor with CFAH turned out to be more effective in comparison with using CFAH only, as evidenced by the additional normalization of TT, the activity of AST, GGT, the contents of bilirubin and fibrinogen, and further correction of the AP activity. It should be noted that the introduction of Essentiale N, Hypoxenum and CFAH reduces de Ritis ratio below the norm, but the use of Heptral and Mexicor with CFAH normalizes it.

The introduction of CFAH proteins with MW more than 130 kDa did not affect hepatocyte biochemical parameters, which had been altered by ALI. The introduction of whole (undivided into fractions) CFAH proteins or CFAH proteins with MW less than 130 kDa additionally, in comparison with CFAH, normalizes the level of FG and the GGT/AST ratio.

A histological examination in the liver tissue of the animals with ALI revealed by discomplexation of lobules the extensive areas of mainly macrovesicular steatosis of hepatocytes in the area of the III and II acini zones, multiple focal necrosis with inflammatory neutrophil-lymphocytic infiltration had developed. An analysis of morphological changes in the liver of the animals with ALI treated with the preparations showed that the correction of disorders which had developed due to hypoxia was most effective when administering allogeneic hepatocytes, Heptral and Mexicor, especially CFAH proteins with MW less than 130 kDa. In the animals of this group, the lamellar structure of the liver was histologically preserved; there was centrolobular microvesicular steatosis of hepatocytes; there was little cell necrosis, small with weak inflammatory lymphohistiocytic infiltration.

ALI suppresses the development of HIR and DHS to SE, as evidenced by a decrease in immune APC number in the spleen, DM and DK in the popliteal regional and contralateral lymph nodes (Fig. 3).

The introduction of Essentiale N with Hypoxenum or Heptral with Mexicor does not affect the formation of HIR, but corrects DHS parameters towards those of the control animals but not to their level. The use of xenogeneic or allogeneic hepatocytes in presence of ALI, in comparison with the previous experimental groups of animals, brought the indices of humoral and cellular forms of immune response closer to the norm values to an even greater extent, but not to the norm yet.

In ALI, the indicators of functional and metabolic activity of circulating neutrophils decrease, with the exception of CAn (it does not change) (Fig. 3).

The administration of Essentiale N and Hypoxenum partially corrects the phagocytic activity of polymorphonuclear leukocytes and some indicators of their oxygen-dependent metabolism (NBT-st o/z and CAo). The use of Heptral and Mexicor, in comparison with Essentiale N and Hypoxenum, reduces IPA, but increases the degree of neutrophil response intermittency to opsonized and non-opsonized zymosan (CO).

The use of XH brings the parameters of neutrophil phagocytosis (PN and IPA) closer to the control parameters. The use of AH turned out to be more effective, since, in comparison with XH, it normalizes the phagocytic activity and changes the oxygen-dependent activity of polymorphonuclear leukocytes towards that of the control animals (normalization of CO and correction of NBT-st n/z and CAo).

The introduction of CFXH corrected the DHS formation, normalized some parameters of neutrophil FMA (FC, NBT-st n/z and o/z), brought other indicators of oxygen-dependent metabolism (PN, IPA, CAn, CAo) closer to the normal values.

The introduction of CFAH turned out to be the most effective, since it normalized most of the investigated parameters of the immune response, of both humoral and cellular forms, with the exception of APC, CAn and CAo, which turned out to be more corrected than when using CFXH.

The introduction of CFAH proteins with MW less than 130 kDa normalizes the indicators of DHS (DM and DK), phagocytic (PC, PN and IPA) and oxygen-dependent metabolic (NBT-sp, NBT-st n/z and o/z, CO) activity of polymorphonuclear leukocytes of peripheral blood and corrects HIR formation (APC) and the values of neutrophil functional backup in response to stimulation with non-opsonized and opsonized zymosan (CAn and CAo). Being administered, CFAH proteins with MW more than 130 kDa did not affect the studied parameters of adaptive and innate immunity (Fig. 3).

In presence of ALI, oxidative stress develops, and LPO processes intensify at the systemic and local levels: in blood plasma and erythrocytes, the concentrations of MDA and AHP increase, whereas the antioxidant defense factors (TAA, SOD and catalase activity) and the level of constant nitric oxide metabolites in plasma decrease.

The use of Essentiale N and Hypoxenum in ALI normalized the activity of catalase in blood plasma, did not affect TAA in erythrocytes and corrected the other studied metabolic parameters at the systemic and local levels towards those of the group of control animals, but not to their level. The use of Heptral with Mexicor, in comparison with Essentiale N and Hypoxenum, normalized TAA, corrected the MDA level in blood plasma and erythrocytes, and normalized the catalase activity in erythrocytes.

The XH administration normalized the SOD activity and additionally corrected the concentrations of AHP and SM_{ON} in te blood plasma, and also normalized TAA in erythrocytes and to a greater degree brought the level of LPO products closer to the control values. When compared with XH, the use of AH additionally corrected the MDA content in plasma and erythrocytes and normalized the SOD activity in erythrocytes.

The use of CFXH normalizes the level of AHP, the SOD activity and corrects to a greater extent the MDA concentration in blood plasma, but in erythrocytes, it normalizes TAA and corrects the MDA content. The administration of only CFAH in presence of ALI, in addition to CFXH effect, corrects the concentrations of MDA and SM_{ON} in blood plasma, normalizes the SOD activity, corrects the AHP level to a greater extent and increases the catalase activity above the normal values. The use of CFAH with Essentiale N and Hypoxenum or Heptral with Mexicor, in addition to CFAH effects in erythrocytes, brings the level of LPO products closer to the values of the control animals.

The introduction of CFAH proteins with MW more than 130 kDa did not affect the activation of LPO, the development of oxidative stress at the general (blood plasma) and local (erythrocytes) levels, or the sorption parameters of red blood cells caused by acute liver ischemia. The introduction of CFAH, undivided CFAH proteins or CFAH proteins with MW less than 130 kDa normalizes the antioxidant defense parameters (the exception was the catalase activity in erythrocytes – correction) and corrects the other studied biochemical parameters at the systemic and local levels.

Thus, when analyzing the impact of the corrective activity of CFAH or CFAH proteins on the development of biochemical markers in acute ischemic liver damage, the formation of humoral and cellular immunity, impaired functional and metabolic activity of peripheral blood neutrophils, endoglobular metabolism, activation of free-radical oxidation according to an ascending efficacy degree, the following sequence was established: lack of such in CFAH proteins with MW more than 130 kDa, the presence of normalizing and correcting activity of CFAH – undivided CFAH proteins – CFAH proteins less than 130 kDa.

Taking into account the widespread distribution and availability of alcoholic beverages and the fact that excessive consumption of ethanol or alcohol surrogates is the main cause leading to the development of terminal stages

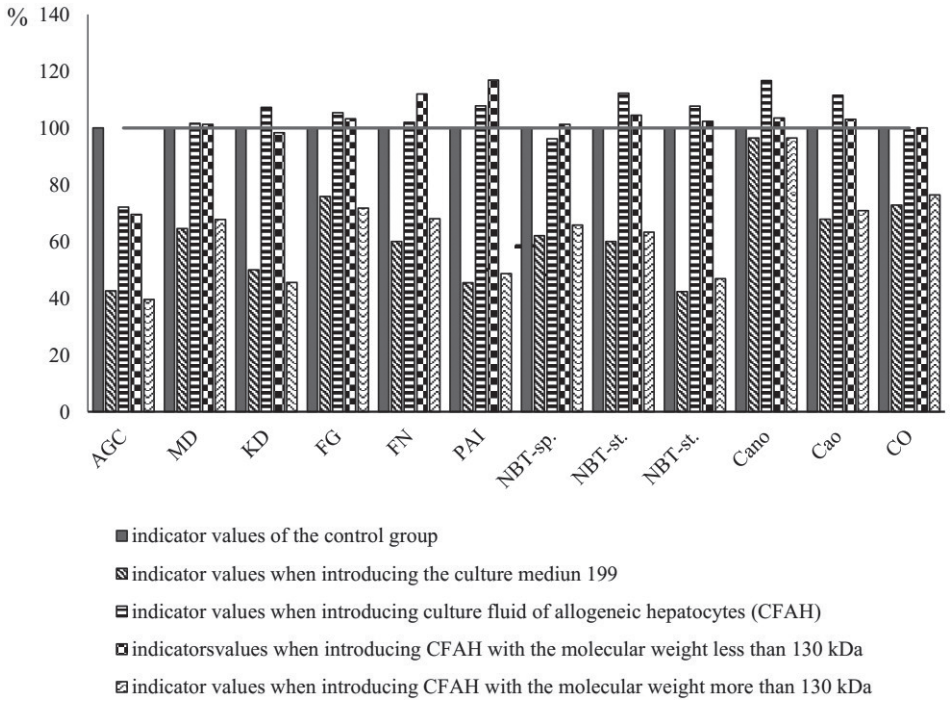

Figure 3. Impact of allogeneic hepatocyte proteins on the development of adaptive immune response and functional-metabolic activity of peripheral blood neutrophils in acute ischemic liver damage. **Note:** APC – antibody-producing cells; DM – difference in the masses of the regional and contralateral lymph nodes; DK – difference in the number of karyocytes in lymph nodes; FG – fibrinogen ; FN – phagocytic number; IPA – index of phagocyte activation ; NBT-test – reduction reaction of nitro-blue tetrazolium; NBT-sp – reduction reaction of nitro-blue tetrazolium, spontaneous; NBT-st – reduction reaction of nitro-blue tetrazolium, stimulated by zymosan; CAn – coefficients of activation to non-opsonized zymosan; CA_O – coefficients of activation to opsonized zymosan; CO – coefficients of opsonization.

of liver disease, it is extremely important to find out the mechanisms of influence of the liver and bone marrow stem cells, hepatocytes, and their waste products in liver pathologies, including those of alcoholic genesis (Gandillet et al. 2005, Got'e et al. 2013, Nutt et al. 2015). At the same time, and analysis of the scientific publications reveals lack of experimental data concerning the influence of cell technologies in conditions of chronic alcohol intoxication (CAI).

In the studies conducted earlier, it was found out that both short-term (5 days) and long-term (30 and 60 days) administrations of ethanol to experimental animals resulted in metabolic disturbances, but if in a short-term ethanol intake, the changes are of reactive character, 30-day and, to a greater extent 60-day ethanol intoxication is manifested by toxic liver damage, oxidative and immune disorders, insufficiently corrected by the use of hepatoprotectors, antioxidants and immunomodulatory agents (Dolgareva et al. 2018).

Functional disorders of hepatocytes due to a long-term 60-day intake of ethanol were characterized by the development of the following biochemical syndromes: cytolysis, intra- and extrahepatic cholestasis, toxic damage to hepatocytes, insufficiency of synthetic and inflammatory processes (increased TT) (Table 4).

The administration of whole proteins of allogeneic hepatocytes (PAH), undivided into fractions, or proteins with the molecular weight (MW) less than 130 kDa to the animals with CAI normalizes the levels of FG, PTI, the AP activity and the GGT/AST ration, brings TT, the activity of AST, ALT, GGT, and the BR concentration closer to the control parameters, but not to reach their values, and does not change the AST/ALT ratios (only with the use of whole PAH, the introduction of proteins with MW less than 130 kDa, the correction of this indicator is observed). In the animals that received PAH with MW more than 130 kDa, neither correction nor normalization of the disturbed biochemical parameters of the hepatocyte functional activity caused by CAI was observed (Table 4).

It has been found that in the animals with CAI, at the systemic (blood plasma) and local (erythrocytes) levels, LPO is activated (an increase in the contents of MDA and AHP), there was oxidative stress (a decrease in TAA, the activity of SOD and the catalase activity), a decrease in the concentration of constant nitric oxide metabolites in blood plasma, and in erythrocytes there was a decrease in sorption properties (a decrease in SCE and SCG) (Table 5).

The introduction of PAH with MW more than 130 kDa did not affect the activation of LPO, the development of oxidative stress at the general (blood plasma) and local (erythrocytes) levels due to the prolonged intake of ethanol. The introduction of undivided PAH or

Table 4. Influence of Proteins of Allogeneic Hepatocytes on Hepatocyte Functional Activity in Chronic Alcohol Intoxication (M±m)

Indicators	Units of measurement	1	2	3	4	5
		Control	Chronic alcohol intoxication and introduction of:			
			–	CFAH proteins	CFAH proteins with MW less than 130 kDa	CFAH proteins with MW more than 130 kDa
AST	U/L	21.5±2.9	47.3±3.4*1	34.5±2.8*1,2	30.3±2.6*1,2	46.4±3.8*1,3,4
ALT	U/L	19.8±1.8	33.5±3.7*1	25.3±1.4*1,2	24.2±2.7*1,2	32.8±4.3*1,3,4
de Ritis ratio, AST/ALT		1.09±0.03	1.41±0.04*1	1.36±0.03*1	1.25±0.04*1,2	1.41±0.03*1,4
GGT	U/L	4.5±0.7	16.5±0.9*1	7.5±0.9*1,2	6.4±0.7*1,2	15.7±1.1*1,3,4
GGT/AST		0.21±0.02	0.38±0.02*1	0.22±0.03*2	0.21±0.03*2	0.34±0.03*1,3,4
AP	U/L	229,3±11.7	356.8±12.1*1	237.3±10.4*2	224.8±9.7*2	361.3±14.6*1,3,4
BR	µmol/L	4.8±0.7	12.2±1.0*1	6.8±0.5*1,2	5.6±0.4*2	11.3±0.9*1,3,4
FG	g/L	4.3±0.2	3.5±0.3*1	4.1±0.2*2	4.8±0.4*2,3	3.3±0.2*1,3,4
PTI	%	67.4±4.1	55.8±2.3*1	64.9±2.2*2	65.9±4.0*2	59.2±2.8*1,4
TT	Un. S-H	1.1±0.08	3.0±0.2*1	2.0±0.14*1,2	1.7±0.2*1,2	3.2±0.2*1,3,4

Table 5. Influence of Proteins of Allogeneic Hepatocytes on Erythrocytes and Blood Plasma Metabolic Parameters in Chronic Alcohol Intoxication (M±m)

Indicators	Units of measurement	1	2	3	4	5
		Control	Chronic alcohol intoxication and introduction of:			
			–	CFAH proteins	CFAH proteins with MW less than 130 kDa	CFAH proteins with MW more than 130 kDa
Blood plasma						
MDA	µmol/L	0.33±0.02	7.1±0.8*1	1.3±0.1*1,2	1.2±0.08*1,2	6.9±0.7*1,3,4
AHP	RU	0.14±0.02	0.9±0.03*1	0.3±0.02*1,2	0.25±0.01*1-3	0.9±0.04*1,3,4
TAA	%	43.9±1.8	34.6±1.3*1	42.4±2.2*2	40.±2.1*2	35.4±1.4*1,3,4
SOD	RU /ml	13.2±0.9	6.5±1.4*1	12.4±1.6*2	14.0±1.3*2	6.7±1.2*1,3,4
Catalase	mcat/L	14.3±1.3	7.0±1.5*1	13.5±2.0*2	14.9±1.8*2	7.7±1.4*1,3,4
SM_{ON}	µmol/L	6.8±0.8	4.0±0.6*1	5.2±0.3*1,2	5.4±0.4*1,2	3.9±0.3*1,3,4
Erythrocytes						
MDA	µmol/L	0.3±0.02	1.1±0.05*1	0.5±0.04*1,2	0.41±0.03*1-3	1.2±0.06*1,3,4
AHP	RU	0.12±0.01	0.47±0.04*1	0.31±0.02*1,2	0.27±0.03*1,2	0.5±0.06*1,3,4
TAA	%	46.2±3.2	35.1±3.3*1	47.1±3.8*2	49.0±4.3*2	37.5±3.3*1,3,4
SOD	RU/ml	27.4±2.2	16.2±1.1*1	25.7±2.4*2	26.5±2.5*2	14.8±2.5*1,3,4
Catalase	mcat/L	10.8±1.3	7.7±0.6*1	12.4±1.5*2	13.0±1.5*2	8.1±0.7*1,3,4
SCE	%	56.4±2.9	29.7±2.0*1	38.6±2.8*1,2	37.1±3.4*1,2	27.9±3.7*1,3,4
SCG	10^{12} gram/ erythrocytes	2.9±0.06	2.0±0.07*1	3.0±0.06*2	3.1±0.07*2	1.9±0.08*1,3,4

proteins with MW less than 130 kDa normalizes the antioxidant defense parameters at the systemic and local levels and brings the content of LPO markers closer to the indicators of the healthy animals, but not to reach their values. In plasma, the concentration of SM_{ON} appeared to be corrected, and in erythrocytes, SCE was normalized, and SCG was corrected (Table 5).

Chronic intake of ethanol results in the suppression of humoral and cellular immune response development to SE (a decrease in immune APCs in the spleen, mass difference in the regional and contralateral popliteal lymph nodes and the number of karyocytes in them), a decrease in the phagocytic activity (a decrease in FC, FN, and IPA) and an increase in the oxygen-dependent metabolic activity of circulating neutrophils (increased NBT-sp, NBT st n/z and o/z, CAn). At the same time, it is important to note a decrease in the functional backup of these cells in response to stimulation with zymosan (CA_O) and a degree of response intermittency to opsonized and non-opsonized zymosan (CO).

The introduction of PAH with MW more than 130 kDa did not affect the changes in adaptive and innate immunity parameters caused by CAI. The use of undivided PAH or proteins with MW less than 130 kDa brings the parameters of HIR and DHS closer to those of the control group, but not to reach their values. As for the oxygen-dependent metabolic activity of circulating neutrophils in the recipients with CAI, the following results were obtained: whole proteins of CFAH correct the values of the phagocytic activity of polymorphonuclear leukocytes, normalize (NBT-sp) and bring the NBT-st n/z, o/z and CO parameters closer to the normal ones, increase the functional backup of these cells in response to stimulation with zymosan. In addition to the action of undivided PAH, proteins with MW less than 130 kDa normalize PN, NBT-st o/z, CAo, and bring PAH, CAn closer to the norm, but do not affect CO.

Thus, prolonged intoxication with ethanol results in a shift from the normal parameters in 100.0% of the studied indicators for metabolic and immune statuses. The data obtained makes it possible to conclude that in the animals in presence of CAI there develop the main biochemical syndromes of liver damage: cytolytic, intracellular cholestasis, toxic damage by immunoinflammatory type and insufficiency of synthetic processes. Besides, in these conditions, there follows oxidative stress, disruption in erythrocyte functional and metabolic activity in circulating blood and formation of innate and adaptive immunity. Undivided CFAH proteins and proteins isolated from them with MW less than 130 kDa turned out to be effective.

The administration of xeno- and allogeneic hepatocytes, their culture fluid, proteins of the culture fluid without and with pharmacological preparations to correct the immunometabolic laboratory parameters in acute experi-

mental liver pathologies of different etiology was expected to raise an issue about their comparative efficacy.

When quantitatively comparing the number of the disrupted laboratory parameters and dividing the severity of disruption by degrees in different experimental conditions, it was found out that in ATLD caused by CTC poisoning, 38.9%, 33.3% and 28.6% of the investigated parameters appeared to be disrupted in III, II and I degrees, respectively. The introduction of Essentiale N and Hypoxenum or Heptral and Mexicor under these circumstances reduces, respectively, the number of impaired indicators of the III degree to 27.8% and 25%, and of the II degree to 22.2% and 25%, which requires additional correction (Zemskov et al. 2005, Uemoto et al. 2016).

The transplantation of FB, XH or AH to the recipients with ATLD reduces the number of impaired indicators of the III degree to 27.8%, 13.9% and 13.9%, and of the II degree to 25%, 27.8% and 16.7%, respectively. The introduction of CFF, CFXH or CFAH within the period of intoxication reduces the number of impaired laboratory parameters of the III degree to 13.9%, 5.6% and 2.8%, and of the II degree to 25%, 13.9% and 0%, respectively. The most effective was the introduction of CFAH with the pharmacological preparations (Essentiale N and Hypoxenum or Heptral and Mexicor), whole CFAH proteins or proteins with MW less than 130 kDa, since only the parameters of the I degree remained altered. The administration of CFAH proteins with MW more than 130 kDa turned out to be completely ineffective.

When comparing the indicators accordging to a degree of disruption severity in acute liver ischemia, it was found out that 18.2%, 57.6% and 27.3% of the studied parameters turned out to be of the I, II and III degrees of disruption, respectively. The introduction of Essentiale N and Hypoxenum or Heptral and Mexicor under these conditions reduces the number of the impaired indicators of the II and III degreess, which require additional correction, to 54.5% and 51.5%, respectively.

The transplantation of FB, XH or AH to the recipients with ALI reduces the number of impaired indicators of the II and III degrees to 44.7%, 42.4% and 24.2%, respectively. The introduction of CFF, CFXH or CFAH during the period of intoxication reduces the number of the impaired laboratory parameters requiring additional correction to 36.3%, 24.3% and 21.2%, respectively. The administration of CFAH with Essentiale N and Hypoxenum or Heptral and Mexicor reduces the number of the impaired indicators of the II and III degrees to 18.1% and 9.0%, respectively. The introduction of CFAH proteins or proteins with MW less than 130 kDa also reduces the number of the impaired indicators of the II and III degrees to 9.1%. The introduction of CFAH proteins with MW more than 130 kDa turned out to be ineffective.

In presence of chronic alcoholic liver damage, the following results were obtained: 31%, 34.5% and 34.5% of the studied immunometabolic parameters were disrupted in the I, II and III degrees, respectively. The transplantation of XH or AH to the recipients with CAI reduces the number of the impaired indicators of the II and III degreesto 37.9% and 24.1%, respectively. The introduction of CFAH in CAI turned out to be the most effective, since it reduced the number of the disrupted laboratory parameters requiring additional correction to 13.8%.

When calculating intrinsic corrective effects of xeno- and allogenic hepatocytes, their culture fluid, proteins of culture fluid without and with the pharmacological preparations to correct the laboratory immunometabolic parameters in ATLD, the results which are presented in Table 6 were obtained.

Proteins of CFAH with th MW more than 130 kDa do not have a corrective activity towards the immunometabolic parameters in ATLD. The proteins of CFAH with MW less than 130 kDa and CFAH when administered in combination with the pharmacological preparation have the highest corrective activity under the conditions of acute toxic liver damage, since the correction score ranged from 92.4 to 100 points. Lower efficacy in ATLD was shown by Essentiale N with Hypoxenum, Heptral with Mexicor, FB and XH (the correction score ranged from 26.9 to 42.2 points). Culture fluids of XH and AH have intermediate efficacy rate in ATLD (Table 6).

CFAH proteins with MW more than 130 kDa do not have a corrective activity towards the immunometabolic parameters in ALI. AH, CFAH, CFAH proteins with MW less than 130 kDa and CFAH when administered in combination with the pharmacological preparations have the greatest corrective activity in presence of acute ischemic liver damage, since the correction score ranged from 71.5 to 89.4 points. Essentiale N with Hypoxenum, Heptral with Mexicor are less effective (the correction score ranged from 35.8 to 39.3 points). FB, XH and their culture fluids have an intermediate efficiency in ALI.

In chronic alcohol intoxication, CFAH has the highest level of corrective activity, followed by AH and XH, the correction scores being 80, 65.1 and 45.1 points, respectively.

According to the literature data and basing on the results obtained, we can summarize that in toxic liver dam-

Table 6. Intrinsic Corrective Effects of Xeno- and Allogeneic Hepatocytes, Their Culture Fluid, Proteins of Culture Fluid Without and With Pharmacological Preparations to Correct Laboratory Immunometabolic Parameters in ATLD

Scheme of correction	Correction score
ATLD and introduction of Essentiale N and Hypoxenum	30.7
ATLD and introduction of Heptral and Mexicor	30.7
ATLD and introduction of xenogeneic hepatocytes	42.2
ATLD and introduction of allogeneic hepatocytes	57.6
ATLD and introduction of CFXH	73.0
ATLD and introduction of CFAH	96.1
ATLD and introduction of CFAH, Essentiale N and Hypoxenum	100
ATLD and introduction of CFAH, Heptral and Mexicor	100
ATLD and introduction of CFAH proteins	92.4
ATLD and introduction of CFAH proteins with MW less than 130 kDa	92.4
ATLD and introduction of CFAH proteins with MW more than 130 kDa	0

age of different etiologies, the neutrophil respiratory burst system is activated, which results in a shift of pro- and antioxidants balance towards weakening the latter ones, that is, towards an increase in LPO processes in cellular membranes, undermining them not only in hepatocytes, but also in erythrocytes (Shikalova et al. 2012, Zabrodskiĭ et al. 2015, Konoplja et al. 2017). The transplantation of xeno- and, especially, allogeneic hepatocytes, to the recipients suffering from ATLD, ALI and CAI limits the processes of free-radical oxidation at the systemic and local (erythrocytes) levels, the systemic inflammatory response at the level of immunity mechanisms, and has significant positive effects on restoring the hepatocyte functional activity and endoglobular metabolism.

There is a large number of experimental and clinical works in the literature on the correction of liver dysfunctions, there are also studies concerning oxidative, immune and erythrocyte disorders and their correction in liver pathologies (Manzini et al. 2013, Thursz et al. 2015, Konoplja et al. 2017, Suyavaran 2017).

Hepatocytes are among the first types of cells which have been used for clinical purposes – cell therapy for patients with congenital and acquired liver failures. They have currently gained a great scientific and practical interest due to the fact that the only way to treat liver failure caused by viral, autoimmune hepatites, hereditary diseases and intoxication is to solve the problems connected with the lack of donor organs (Liu et al. 2009, Evseeva et al. 2021).

The existing problems in this area that require further solution are the characteristics of a state of the introduced cells, the methods of their introduction, and determination of the number of the introduced cells (Gandillet et al. 2005, Jabri et al. 2018). At the same time, the mechanisms of metabolic correction of the implanted hepatocytes require further research. The isolation of the "active" principle from the culture fluid of hepatocytes is promising. The reasons for this are the facts earlier obtained in our laboratory and proving that not only the transplantation of allogeneic intact hepatocytes, but also the introduction of culture fluid obtained on their basis to the recipients suffering from experimental acute liver hypoxia, acute toxic hepatitis caused by carbon tetrachloride and prolonged alcohol intoxication significantly reduces the development of immune-inflammatory syndrome in the liver, normalizes hepatocytes synthetic function, prevents the development of oxidative stress and innate immunity impairment (Konoplya et al. 2016, Razumova et al. 2016).

The problem of pharmacological therapy for liver diseases is far from being solved, and poor results of treating acute and chronic liver damage leading to the development of liver failure are largely associated with lack of effective pathogenetic therapy, and therefore a promising direction in the treatment of such conditions is the use of cellular technologies (Manzini et al. 2013, Reiling et al. 2015, Donadon et al. 2016, Warzecha et al. 2017), including the use of isolated xeno- and allogeneic hepatocytes. For the time being, the mechanism of action of hepatocytes used to correct disorders in hepatopathies cannot be considered adequately explained. It is difficult to imagine that the therapeutic effect is associated with the organ-replacing function of the cells being transplanted (Liu et al. 2009). It is likely to be due to humoral and molecular mechanisms responsible for activation and regeneration of the recipient's hepatocyte functional activity by means of producing regulatory peptides (Chikoteev SP et al. 2003, Shalakhmetova et al. 2009, Reiling et al. 2015, Danilova et al. 2020).

Thus, in patients with acute forms of liver damage – acute viral hepatitis A and B with icteritous form of a mild and moderate course, immune-stimulating factors with MW of 55–65 and 10–15 kDa were revealed in blood plasma by gel chromatography on a Sephadex G-100, and whereas one factor with MW of 55–65 kDa was contrykal sensitive (that is, had a proteolytic activity), the other factor with low molecular weight turned out to be contrykal resistant and contained, in addition to the peptide part, an RNA fragment. In blood serum of patients suffering from chronic active and persistent hepatitis, there was also found an immune-stimulating factor with a low molecular weight, in contrast to the serum of patients suffering from liver cirrhosis. Due to the experiments on rats, we have found out that low-molecular-weight humoral factors of animals' serum with acute toxic liver damage caused by the introduction of carbon tetrachloride have some properties to enhance the proliferation of hepatocytes in addition to the immune-stimulating activity. When cultivating embryonic liver cells, non-parenchymatous cells of the organ (Kupffer, Ito), macrophages, and splenocytes produce hepatocyte growth factor (HGF, cytokine FS, scatter factor) of a glycoprotein nature consisting of α and β chains and with MW of 58–69 and 30–34 kDa, respectively. HGF is a strong mitogen for hepatocytes and is involved in the liver regeneration process, stimulates the proliferation of some types of epithelial cells, vascular epithelial cells and melanocytes, and protects hepatocytes from cold injury. Human HGF is homologous to feline, murine, rat and porcine hepatocyte growth factor. In the experiment with acute liver failure (toxic damage, ischemia, liver resection), there was a rapid increase in the growth factor level during the early phase of liver regeneration; at the same time while angiogenesis, cell proliferation and migration were induced, apoptosis was inhibited, and the development of post-inflammatory fibrosis was inhibited as well, but nonspecific tolerance increased (Sugiura et al. 2013). Serine proteinase inhibitor Kazal type 3 (SPINK3) is capable of improving cell proliferation, including hepatocytes after partial liver resection. Structurally, it is identical to epidermal growth factor (EGF), which is a polypeptide with MW of 6054 kDa (Chang et al. 2016). Each of the listed low-molecular-weight polypeptide factors can be an active principle in the obtained and studied protein fraction of hepatocytes in newborn mice and rats with MW less than 130 kDa. This fraction can contain active hepatoprotective, immunomodulating proteins that are not related to those described above, which predetermines the prospect of isolating specific proteins and creating a drug on this basis, followed by preclinical testing on experimental models of liver pathology.

Conclusion

1. Introduction of xenogeneic or allogeneic hepatocytes, their culture fluid, Essentiale N with Hypoxenum or Heptral with Mexicor to the animals having no evidence of disease does not cause significant morphological changes in the liver, parameters of functional and metabolic activity of hepatocytes, neutrophils and erythrocytes of circulating blood, indicators of systemic metabolism and adaptive immunity.
2. In case of toxic liver damage caused by five-fold introduction of carbon tetrachloride or ethanol for 60 days, acute ischemic damage, there developed the main biochemical syndromes of liver damage (cytolysis, intrahepatic and extrahepatic cholestasis, intracellular cholestasis with jaundice and toxic damage of hepatocytes, deficiency of synthetic and immuno-inflammatory processes), impaired formation of immune response, functional and metabolic activity of polymorphonuclear leukocytes, development of oxidative stress, intensification of lipid peroxidation processes at the systemic (blood plasma) and local levels (erythrocytes).The intensity of changes depends on the etiological factor of liver damage.
3. The introduction of hepatocytes from newborn rats, to a greater extent of culture fluid obtained on their basis, to allogeneic recipients with toxic liver damage more effectively corrects morphological changes and biochemical parameters of hepatocyte functional activity than that of xenogeneic hepatocytes, their culture fluid and the combination of Essentiale N and Hypoxenum or Heptral and Mexicor.
4. The introduction of hepatocytes, culture fluid obtained on their basis, to allogeneic recipients with acute toxic hepatopathy more effectively corrects the formation of adaptive and innate forms of immunity, metabolic systemic and local disorders due to the exposure to carbon tetrachloride in comparison with xenogeneic hepatocytes, their culture fluid, and the pharmacological preparations.
5. Introduction of hepatocytes from intact newborn rats, their culture fluid to allogeneic recipients with acute liver ischemia in a greater degree corrects morphofunctional changes in the liver, disorders in adoptive and innate immunity formation, systemic and local metabolic changes arising from acute liver ischemia in comparison with the combinations of Essentiale N and Hypoxenum or Heptral and Mexicor, fibroblasts, xenogeneic hepatocytes, or culture fluid of xenogeneic hepatocytes.
6. In the context of chronic alcohol intoxication, transplantation of xenogeneic or allogeneic hepatocytes normalizes 20.7% and 44.8% out of 96.7% of the altered laboratory parameters of metabolism, respectively. The administration of culture fluid of allogeneic hepatocytes turned out to be more effective as it normalized 69% of the immunometabolic parameters.
7. Hepatoprotective, immunomodulation factors of peptidic nature with MW less than 130 kDa were revealed in the supernatant of hepatocytes from newborn rats.

Recommendations

1. To further purify proteins in culture fluid of allogeneic hepatocytes with the determination of hepatoprotective, metabolic, immunomodulation effects in the factors of peptidic nature.
2. To develop a pharmacological preparation(s) based on the revealed factor(s) of peptidic nature of culture fluid of allogeneic hepatocytes, followed by preclinical trials on experimental models of liver pathology.
3. To apply the knowledge about the pharmacological properties of allogeneic hepatocytes, their culture fluid, proteins of culture fluid of allogeneic hepatocytes in context of acute and chronic damages to the liver in the educational process at medical universities.

Conflict of interest

The authors declare no conflict of interests.

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