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STUDY OF PHARMACOLOGICAL ACTIVITY IN SUBLIMATED CULTURE LIQUID OF ALLOGENIC, XENOGENEIC HEPATOCYTES AND FIBROBLASTS FOR THE CORRECTION OF LIVER DAMAGES IN CARBON TETRACHLORIDE INTOXICATION

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Abstract

Introduction: At the present time many clinical and experimental studies are devoted to the correction of hepatocytes and liver functional abnormalities with the use of cellular technologies.

Materials and Methods: Experimental studies were performed on 175 Wistar rats weighing from 120 to 140 g. Acute toxic liver damages (ATLD) in laboratory animals was simulated by intramuscular injection of carbon tetrachloride (CTC) at a dose of 3 ml/kg as 50% solution in olive oil five times with an interval of 24 h.

Results and Discussion: The introduction of Essentiale H and hypoxen to the animals with ATLD enabled to normalize PTI, TAA and the activity of antioxidant defense enzymes (SOD and catalase) and corrected the concentrations of MDA and SM_{ON}. Simultaneous introduction of CLAH proteins, Essentiale H and hypoxen to animals with acute toxic liver damage, in comparison with the previous groups of experimental animals, additionally normalizes the concentration of MDA and SM_{ON} in blood plasma. Combined administration of Essentiale N, hypoxen and CLAH proteins, in comparison with the previous groups of experimental animals, normalizes the concentration of MDA, SOD activity, sorption capacity of erythrocytes.

Conclusion: The administration of hepatocytes culture liquid of intact rats in combination with pharmacological preparations (Essentiale Forte H and Hypoxen) to allogeneic recipients intoxicated with CTC corrects systemic metabolic disorders arising from the action of hepatotrophic poison more efficiently, in comparison with their separate use.

Keywords: allogenic hepatocytes, xenogeneic hepatocytes, fibroblasts, sublimated culture liquid, liver damages in carbon tetrachloride intoxication, Essentiale forte H, Hypoxen.

Introduction

The incidence of acute and chronic liver diseases in the overall structure of human diseases and the mortality from this type of pathology keeps steadily growing even in economically developed countries [1, 2, 3]. The problems of diagnostics, pathogenesis and treatment of chronic and acute liver diseases remain relevant these days. This is due not only to the continuing tendency for increase in the number of patients with this nosology, but the diagnostics complexity and the difficulty choosing the most effective therapies in this category of patients as well. Frequent liver pathologies include acute toxic liver damages (ATLD), complicated by the development of hepatic insufficiency under intoxication with hepatotoxic poisons, taking large doses of analgesics, anti-inflammatory, antibacterial, antimetabolic and other medications [4, 5, 6].

Unsatisfactory results of ATLD treatment are currently due to the lack of effective

pathogenetically substantiated pharmacotherapy, that is why, a promising direction for such pathological conditions correction is in the use of cellular technologies, and one of such directions is the use of humoral factors obtained during the cultivation of xeno- and allogeneic hepatocytes, fetal fibroblasts [7, 8, 9]. This is due to the prevailing opinion that the effect of whole cells transplantation is associated not so much with their organ-substituting function as with the humoral and molecular mechanisms responsible for increasing not only the activity of recipient's hepatic cells (hepatocytes), but also their regeneration due to the induction of regulatory peptides production, such as growth factor [10, 11, 12].

Research studies conducted during recent years confirm that the damage and regeneration of hepatocytes by different physical, chemical and biological factors are predominantly caused by immune, oxidant disorders and structural-functional changes in the properties of red blood cells, which, in their turn, are a kind of "cellular dosimeter" of pathogenic endo- and/or exogenous factors action [13, 14, 15]. At the present time many clinical and experimental studies are devoted to the correction of hepatocytes and liver functional abnormalities with the use of cellular technologies, there are a few works on the study of oxidative, immune and erythrocytic disorders and their pharmacological and non-pharmacological correction in various types of liver pathology, but there are practically no researches studying humoral factors in culture liquid of xeno- and allogenic hepatic cells (hepatocytes) [16, 17, 18]. Analyzing the fractions of humoral factors contained in the liquid after xeno- and allografts cultivation in it seems to be a promising direction, since it is them that are the active principles in the repair of liver cells [19, 20, 21, 22].

Aim of the research: to estimate the proteins efficacy in culture liquid of xeno-, allogeneic hepatocytes, and fibroblasts in the correction of metabolic and morphological disorders in acute toxic liver damage.

Materials and Methods

Experimental studies were performed on 175 Wistar rats weighing from 120 to 140 g. Experiments included rats which underwent the quarantine regimen of Kursk State Medical University vivarium, which had no signs of somatic and infectious diseases. In addition, 65 hepatocytes donors were involved 5-6 days after birth, including 30 Wistar rats and 35 white mice. All the investigations were carried out at the same time of day, from 8 am to 12, subjected to all the principles set out in the Convention for the Protection of Vertebrate Animals used for experimental and other purposes (Strasbourg, France, 1986) and according to the rules of laboratory practice in the Russian Federation (Directive of the Ministry of Health of the Russian Federation No. 267 of June 19, 2003).

ATLD in laboratory animals was simulated by intramuscular injection of carbon tetrachloride (CTC) at a dose of 3 ml/kg as 50% solution in olive oil five times with an interval of 24 h (Smakhtin M.Yu. et al., 2003). Xenogeneic (murine) and allergenic hepatocytes (XH, AH) were isolated from animals 5-6 days after birth by M.N. Berry, D.S. Friend method (1969).

To get culture liquid of xenogeneic and allogeneic hepatocytes (CLXH, CLAH), $5x10^7$ cells per 3 ml of medium were cultivated in medium 199 for 6 hours. Following the incubation period termination, the cells were precipitated by centrifugation (15 minutes at 400 g). Protein concentration in culture liquid was determined by the Kjeldahl method. The obtained CLXH and CLAH were administered intraperitoneally five times with the first injection of hepatotrophic poison (with a 24-hour interval) to the rats with ATLD in the amount of 5 m/ kg of protein.

FB were isolated from corpora and extremities fragments of 8-12-week-old human abortus tissues by their mechanical disintegration to microfragments of 0.1-0.2 mm and further cultivation of cells. The resulting culture liquid of FB (CLFB) was introduced intraperitoneally to rats with ATLD in the amount of 5 mg/kg, simultaneously with the first injection of CTC.

To obtain proteins from 50-100 ml of culture liquid, they were precipitated with an equal volume of 10% trichloracetic acid; the resulting precipitate was separated by 20

minutes centrifugation at 1500 g. After supernatant separation, the precipitate was diluted in normal saline and dialyzed with two changes of phosphate-buffered saline pH 7.2-7.4 for 18 hours.

After the protein concentration had been determined and brought by 0.9% solution of sodium chloride to 5 mg/ml, the obtained solution was filtered through sterilizing membranes of 0.2 μm, packed in sterile 2 ml vials and lyophilized in a freeze drying plant "VIRTIS".

The obtained proteins of CLXH, CLAH or KLFB were intraperitoneally introduced with the first injection of hepatotrophic toxin five times (with a 24-hour interval) to the rats with ATLD in the amount of 5 mg/kg of protein.

Poisoning with hepatotrophic toxin at the doses used and frequency of administration according to literature data and in our experiments did not result in their death within the experiment, the animals were removed from the experiment in 24 hours after the last administration of CTC, culture liquid and their proteins AH, FB, XH, essentiale forte H and hypoxen.

The study groups included 12-14 animals; the control group included 19 healthy rats of the same age, sex and body weight (Table 1).

Table 1

Groups of animals

Groups of animals	Amount
Intact	19
CTC	14
CTC + culture fluid of allogeneic hepatocytes	12
CTC + culture fluid of xenogeneic hepatocytes	14
CTC + culture liquid of fibroblasts	13
CTC + proteins of culture fluid of allogeneic hepatocytes	14
CTC + proteins of culture fluid of xenogeneic hepatocytes	12
CTC + proteins of culture liquid of fibroblasts	14
CTC + proteins of culture fluid of allogeneic hepatocytes after lyophilization	13
CTC + proteins of culture fluid of xenogeneic hepatocytes after lyophilization	14
CTC + proteins of culture liquid of fibroblasts after lyophilization	14
CTC + essentiale + hypoxen	12
CTC + culture fluid of allogeneic hepatocytes + essentiale + hypoxen	14
Total sum:	175

Functional status evaluation of the liver cells (hepatocytes) was carried out by the determination in the blood serum of alanine aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase, prothrombin index activities, thymol turbidity test using standard reagent set.

Liver enzymes activity was evaluated by means of automatic biochemical analyzer Vitalab Flexor E (the Netherlands) with Analyticon® Biotechnologies AG reagents (Germany). Fibrinogen content was determined by means of a semi-automatic analyzer of hemostatic profile STart4 (France), reagents Diagnostica Stago (France).

The intensity of lipid peroxidation processes (LPO) was assessed by acyl hydroperoxides (AHP) and malonic dialdehyde

(MDA) content in the blood plasma and erythrocytes. Antioxidant system state was determined by direct/ competitive solid-phase enzyme immunoassay (EIA) with the detection of reaction products in the wave-length spectrum of 405-630 using ready-made commercial kits: superoxide dismutase activity (SOD) "Bender Medsystems" (Austria) and catalase "Cayman Chemical" (the USA). Total antioxidative activity (TAA) was determined by a method based on the degree of inhibition of ascorbate- and ferro-induced oxidation of Tween-80 to MDA. The level of stable metabolites of nitric oxide (SM_{NO}) was detected using two analytical procedures: endogenous nitrite measurement and nitrate conversion into nitrite using nitrite reductase followed by total nitrite measurement by azo dye absorption in Griess test at wave-length of 540 nm using a kit for solid-phase EIA of "R & D" firm (England). All the results of the enzyme-linked immunoassay were recorded by means of automatic reader for the EIA –Efos 9305" (Russia).

The total number of peripheral blood erythrocytes and their hemoglobin content were calculated according to generally accepted standard methods. To this end, heparinized blood was centrifuged, plasma and erythrocytes were obtained. General sorption capacity of erythrocytes, due to the external architectonics of cell membrane and the sorption capacity of erythrocytes glycocalyx for alcian blue were determined.

Phagocytic activity of polymorphonuclear leukocytes was assessed by the following indices: phagocytic index (PI), phagocytic number (PN) and index of phagocytosis activity (IPA). The activity of oxygen-dependent systems of polymorphonuclear leukocytes was evaluated spectrophotometrically by the reaction of nitro blue tetrazolium reduction (NBT-test) with the use of PD 303 S Apel (Japan) spectrophotometer.

After animals had been removed from the experiment, the liver was taken for morphological study. Pieces of the studied organs were fixed in 10% neutral formalin. We used tissues embedding in paraffin, partly in gelatin; in some cases, non-fixed freshly frozen slices were prepared in thermostatic cooler. Further on the microtome, paraffin slices of 5-7 μm were prepared; they were subjected to paraffin removing, followed by staining with hematoxylin and eosin. Cryostat sections were stained with Sudan IV.

Alongside with routine histological methods of investigation, a morphometric research was used to morphologically evaluate the liver tissue condition on histologic sections, allowing to objectively estimate the degree and peculiarities of ongoing processes in hepatic parenchyma and its potential regenerative capabilities.

Statistical processing of the results obtained in the thesis research was conducted using the criteria of variational and statistical analysis with the calculation of mean values (M), arithmetic means error (m). The significance of differences was evaluated according to Mann-Whitney U-test. Differences with $p < 0.05$ were considered statistically significant.

Results and Discussion

Intoxication with CTC for 5 days results in the development of the following biochemical syndromes of hepatic tissue damage: cytolysis syndrome (an increased activity of AST, ALT, which, in its turn, reduces De Ritis ratio), the syndromes of extrahepatic and intrahepatic cholestasis (increased GGT and APT activity) and toxic hepatocytes damage (an increase in bilirubin concentration, AST, ALT, GGT, APT activity, GGT /AST rate, and De Ritis ratio becomes less than 1), decline in synthetic processes (reduction of PTI and FB) and inflammatory syndrome (an increase in TTT) (Table 2).

The introduction of the culture medium 199, on the basis of which the culture liquid of fibroblasts was obtained, to the animals intoxicated with CTC does not affect the parameters of hepatocytes functional activity altered with the administration of hepatotrophic poison (Table 2).

The use of CLFB under ATLD conditions normalizes the GGT/AST rate, partially corrects the activity of APT, ALT, GGT, AST /ALT rate, GGT /AST rate, bilirubin and fibrinogen concentrations towards the parameters of healthy animals (Table 2).

Table 2

Function of hepatocytes, the plasma oxidative status, and metabolic status in erythrocytes and FMA of peripheral blood neutrophils in acute toxic hepatopathy when transplanting the culture liquid of fibroblasts (M ± m)

Indicators	Unit measure	1	2	3	4
		Control	Introduction of CTC and:		
			–	Medium 199	CLFB
AST	U/l	28.9±2.3	53.9±4.2*1	50.1±4.2*1	51.2±4.0*1
ALT	U/l	22.2±1.8	88.3±4.9*1	83.2±5.4*1	60.8±4.7*1-3
APT	U/l	232.1±16.8	458.2±34.1*1	401.3±17.2*1	306.7±16.1*1-3
GGT	U/l	5.8±0.2	20.1±2.1*1	19.5±1.4*1	8.9±0.6*1-3
Bili	umol/l	5.3±0.3	19.1±1.1*1	17.5±0.4*1	10.9±0.9*1-3
PTI	%	61.9±3.7	45.9±3.2*1	43.5±2.9*1	47.8±3.1*1
FBG	h/l	4.2±0.1	2.2±0.03*1	2.3±0.05*1	3.4±0.02*1-3
TTT	Unit S-H	2.4±0.3	4.2±0.1*1	4.1±0.2*1	4.0±0.3*1
MDA	umol/l	2.1±0.05	5.7±0.3*1	5.3±0.2*1	3.4±0.2*1-3
AHP	standard unit	0.8±0.03	2.0±0.1*1	1.9±0.05*1	0.94±0.03*1-3
SOD	stand.U/ml	10.9±0.7	8.3±0.6*1	8.1±0.4*1	9.2±0.5*1-3
TAA	%	39.2±1.0	32.9±1.9*1	33.1±2.6*1	41.0±3.2*2.3
Kat	μkat/l	11.9±0.5	9.1±0.7*1	8.9±0.4*1	11.8±1.2*2.3
SM_{ON}	umol/l	6.7±0.3	2.8±0.07*1	2.9±0.1*1	3.8±0.2*1-3
MDA	umol/l	0.3±0.02	0.6±0.03*1	0.7±0.07*1	0.3±0.02*2.3
AHP	stand.U	0.13±0.02	0.5±0.02*1	0.6±0.08*1	0.2±0.02*1-3
SOD	stand.U/ml	24.4±1.6	8.9±0.1*1	8.5±0.4*1	16.7±0.8*1-3
SCE	%	50.5±1.8	28.5±3.5*1	29.1±2.7*1	48.7±2.5*2.3
SCG	10^{12} g/eryth.	2.8±0.04	1.7±0.05*1	1.6±0.1*1	2.4±0.08*1-3
Erythrocytes count	10^{12} /l	4.2±0.07	3.2±0.03*1	3.3±0.07*1	4.1±0.2*2.3
Hemoglobin	h/l	14.5±0.4	13.5±0.4*1	13.2±0.3*1	14.3±0.5*2.3
NBT – sp.	mOD	0.7±0.05	1.1±0.03*1	1.2±0.06*1	0.8±0.05*2.3
NBT-ind. n/z	mOD	0.9±0.05	1.6±0.05*1	1.7±0.05*1	1.3±0.06*1-3
NBT-ind. o/z	mOD	1.1±0.06	1.5±0.05*1	1.6±0.06*1	1.6±0.05*1
PI	absolute	47.5±1.0	74.8±2.6*1	72.7±1.9*1	52.8±1.7*1-3
PN	absolute	2.0±0.1	2.7±0.05*1	2.6±0.1*1	2.7±0.1*1

Note. The asterisk marked significant differences of average arithmetical ($p < 0.05$); figures close to the star are in relation to that of a group of these differences.

When evaluating oxidative parameters of the blood plasma in experimental animals with ATLD, without and by the introduction of the culture medium 199, the activation of LPO processes (an increase in MDA and AHP levels), a decrease in antioxidant defense indices (TAA), activity of SOD and Kat) and SM_{ON} content (Table 2) have been revealed.

The use of CLFB, in contrast to the indices in the 2nd and 3d groups of animals, completely restores TAA, catalase activity and corrects, but not to the control level, the concentration of lipid peroxidation products and stable metabolites of nitrogen oxide and SOD activity (Table 2).

Having analyzed the indices of metabolic activity in red blood cells, we found out that the introduction of hepatotoxic poison to experimental animals reduces their total count, hemoglobin content, increases endoglobular processes of lipid peroxidation (an increase in MDA and AHP concentrations), reduces SOD

activity and sorption indexes (SCE and SCG) (Table 2).

The introduction of the culture medium 199 does not affect the parameters of erythrocytes metabolic status, altered by the intake of CTC. The use of CLFB normalizes the total count of erythrocytes, the level of hemoglobin in them, MDA concentration, the total sorption capacity of erythrocytes (SCE), and corrects, but not to the parameters of healthy rats, the activity of SOD, the level of AHP, the sorption capacity of glycocalyx in red blood cells (SCG) (Table 2).

The study of innate immunity factors gave the following results. In rats with ATLD, without or by the introduction of the culture medium 199, an increase in all the studied indices of the functional-metabolic activity of peripheral blood neutrophils was revealed, i.e., oxygen-dependent (an increase in NBT – sp., NBT – ind. n/z, NBT – ind. o/z) and phagocytic activity (an increase of PI, PN and EIA) (Table 2).

The introduction of CLFB normalized the NBT-sp. and corrected NBT – ind. n/z, PI and EIA towards the parameters of healthy animals (Table 2).

During histologic examination of liver tissues of animals intoxicated with carbon tetrachloride, extensive areas of centrolobular, large-drop adipose degeneration of hepatocytes, multiple focal necrosis with inflammatory neutrophilic-lymphocyte infiltration predominantly, and disturbances in beam structure of lobules were detected.

Microscopically, in animals with ATLD receiving CLFB, there is a disturbance in liver histoarchitecture, leukocyte-lymphocyte infiltration, and centrolobular large-drop adipose degeneration. In the center of the lobule plethora is observed.

The use of culture liquid of xeno- and allogeneic hepatocytes for metabolic disorders correction in acute toxic hepatopathy. The use of CLXH in animals with CTC poisoning, in comparison with the group of rats with ATLD only, normalizes the activity of GGT and APT, corrects the other hepatotrophic poison-induced parameters, characterizing functional-metabolic activity of hepatocytes, towards the control group of animals (Table 3).

The CLAH is more effective, as in comparison with the CLXH, its use under the conditions of ATLD further normalizes the activity of the AST, GGT/AST and PTI rate, corrects ALT activity, De Ritis ratio and FBG content (Table 3).

The study of oxidant status at the systemic level showed that the use of CLXH normalizes TAA and catalase activity and corrects, but not up to the parameters of healthy animals, the level of LPO products and the activity of SOD (Table 3).

Introduction of CLAH, in comparison with the previous group of animals, additionally normalizes the concentration of AHP, SOD activity, corrects the concentration of SM_{NO} to a greater extent (Table 3).

The study of erythrocytic parameters revealed that the use of CLXH normalized the total number of erythrocytes, the level of hemoglobin in them and corrected the parameters of LPO and the sorption capacity of red blood cells towards the control group, the activity of SOD as well (Table 3).

The use of CLAH in animals under the conditions of CTC poisoning, in addition to the effects of CLXH, normalizes SCG, MDA level and corrects to a greater degree SCE and SOD activity of peripheral blood erythrocytes (Table 3).

Further, it was found that compounds with the corrective effect on the function of peripheral blood polymorphonuclear leukocytes are extracellular, since the introduction of CLXH to intoxicated recipients normalized cell activity rate KAo and correlated, but not to the control values, the other studied indices of neutrophils FMA (Table 3).

The administration of CLAH, in comparison with the CLXH, additionally normalizes NBT-sp., NBT-ind. o/z and normalizes and corrects to a greater degree the phagocytic activity of polymorphonuclear leukocytes of peripheral blood (Table 3).

The nature of morphological liver changes in animals with ATLD, which received culture liquid of XH is basically similar to the pathological morphology of the liver tissue that occurs in rats with ATLD: large-drop adipose degeneration of hepatocytes in the central parts of lobes, necrotic changes with inflammatory

cellular infiltration and discomplexation of lobules remain unchanged.

Table 3

Function of hepatocytes, plasma oxidative status, and metabolic status of erythrocytes and FMA of peripheral blood neutrophils in acute toxic hepatopathy under the transplantation of culture liquid of xenogeneic and allogeneic hepatocytes (M ± m)

Indicators	Unit measure	1	2	3	4
		Control	Introduction of CTC and culture liquid of: –	Xenogeneic hepatocytes	Allogeneic hepatocytes
AST	U/l	28.9±2.3	53.9±4.2*1	40.1±2.1*1.2	30.3±2.6*2.3
ALT	U/l	22.2±1.8	88.3±4.9*1	53.0±3.5*1.2	34.8±3.2*1-3
APT	U/l	232.1±16.8	458.2±34.1*1	262.3±14.1*2	263.3±11.1*2
GGT	U/l	5.8±0.2	20.1±2.1*1	0.76±0.04*1.2	0.87±0.06*1-3
Bili	umol/l	5.3±0.3	19.1±1.1*1	6.7±0.3*1.2	6.3±0.4*1.2
PTI	%	61.9±3.7	45.9±3.2*1	53.2±3.3*1.2	56.1±3.0*2.3
FBG	h/l	4.2±0.1	2.2±0.03*1	3.3±0.04*1.2	3.8±0.1*1-3
TTT	Unit S-H	2.4±0.3	4.2±0.1*1	3.1±0.05*1.2	3.0±0.05*1.2
MDA	umol/l	2.1±0.05	5.7±0.3*1	3.2±0.1*1.2	3.1±0.06*1.2
AHP	standard unit	0.8±0.03	2.0±0.1*1	0.9±0.05*1.2	0.74±0.04*2.3
SOD	stand.U/ml	10.9±0.7	8.3±0.6*1	9.9±0.6*1.2	12.0±0.5*2.3
TAA	%	39.2±1.0	32.9±1.9*1	42.6±2.6*2	43.8±3.3*2
Kat	µkat/l	11.9±0.5	9.1±0.7*1	12.6±0.7*2	12.4±1.1*2
SM_{ON}	umol/l	6.7±0.3	2.8±0.07*1	2.7±0.1*1	4.9±0.1*1-3
MDA	umol/l	0.3±0.02	0.6±0.03*1	0.4±0.04*1.2	0.3±0.02*2
AHP	stand.U	0.13±0.02	0.5±0.02*1	0.2±0.01*1.2	0.21±0.01*1.2
SOD	stand.U/ml	24.4±1.6	8.9±0.1*1	14.3±0.7*1.2	19.8±1.3*1-3
SCE	%	50.5±1.8	28.5±3.5*1	36.2±3.2*1.2	44.2±2.2*1-3
SCG	10^{12} g/eryth.	2.8±0.04	1.7±0.05*1	2.5±0.2*1.2	2.7±0.08*2
Erythrocytes count	10^{12} /l	4.2±0.07	3.2±0.03*1	4.1±0.2*2	4.2±0.2*2
Hemoglobin	h/l	14.5±0.4	13.5±0.4*1	14.5±0.4*2	14.7±0.8*2
NBT – sp.	mOD	0.7±0.05	1.1±0.03*1	0.9±0.06*1.2	0.8±0.06*2
NBT-ind. n/z	mOD	0.9±0.05	1.6±0.05*1	1.3±0.05*1.2	1.2±0.05*1.2
NBT-ind. o/z	mOD	1.1±0.06	1.5±0.05*1	1.4±0.04*1.2	1.2±0.04 *2.3
PI	absolute	47.5±1.0	74.8±2.6*1	58.7±2.0*1.2	51.1±2.5*1-3
PN	absolute	2.0±0.1	2.7±0.05*1	2.4±0.06*1.2	1.8±0.04 *1-3

Note. The asterisk marked significant differences of average arithmetical ($p < 0.05$); figures close to the star are in relation to that of a group of these differences.

In animals with ATLD receiving CLAH, in contrast to the group of animals with ATLD without correction, histoarchitecture of the liver tissue is morphologically basically preserved, but in the central sections of the lobules there is a fine-focal predominantly small-drop adipose degeneration of hepatocytes, necrotic changes were not detected.

The use of proteins of culture liquid of fibroblasts, xenogeneic and allogeneic hepatocytes in correction of metabolic disorders in acute toxic hepatopathy. The introduction of CLFB proteins to experimental rats with ATLD prior or after their lyophilization, in comparison with the group intoxicated with CTC, normalizes GGT/ AST rate, TAA and catalase activity, corrects, but

not to the level of healthy animals, ALT, APT, GGT, SOD, bilirubin and fibrinogen concentrations, indicices of LPO and SM_{ON}.

Proteins of CLXH (prior and after lyophilization) turned out to be more effective, since their introduction to animals with ATLD normalized TAA, the activity of alkaline phosphatase, GGT, SOD, catalase, prothrombin index and brought all the other studied parameters of functional-metabolic activity of hepatocytes and oxidant status closer to the normal ones.

The proteins of CLAH appeared to have the greatest efficacy in normalization and correction of metabolic disorders caused by hepatotrophic poisoning. When being used in animals with ATLD, ALT activity, De Ritis ratio, bilirubin and fibrinogen concentrations, MDA, SM_{ON} and thymol turbidity test are corrected in the blood plasma, while all other studied parameters of hepatocyte function and oxidant status are normalized. Statistically significant absence of differences, when introducing CLAH, proteins without and after lyophilization of CLAH to those intoxicated with hepatotrophic poison, ought to be remarked .

The introduction of Essentiale H and hypoxen to the animals with ATLD enabled to normalize PTI and correct, but not up to the standard, the other studied parameters characterizing the functional activity of hepatocytes (Figure).

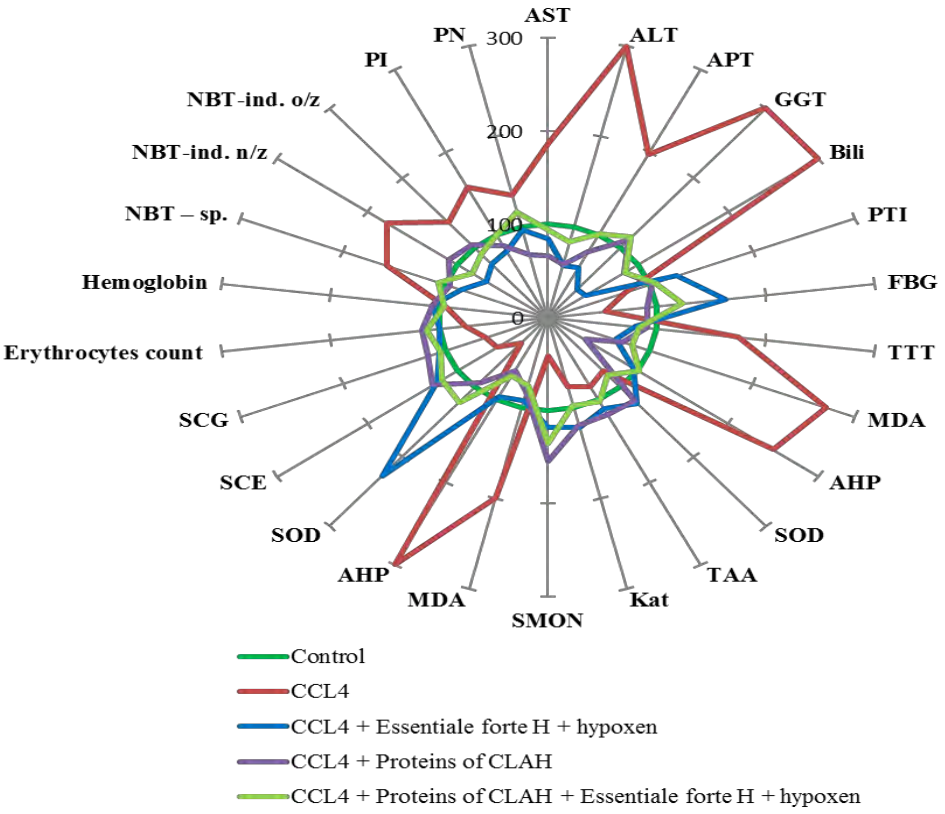

Fig. Influence of proteins of culture liquid of allogeneic hepatocytes, essentiale forte H and hypoxen on hepatocyte function, plasma oxidative status, metabolic status of erythrocytes and FMA of peripheral blood neutrophils in acute toxic hepatopathy

Combined introduction of CLAH proteins and intoxication with CTC normalizes the activity of AST, GGT, APT, GGT/ AST rate, PTI and corrects, but not up to the norm, other studied parameters reflecting the functional-metabolic activity of liver cells (Figure).

The simultaneous use of CLAH and pharmacological agents in animals with acute toxic liver damage normalizes, with the exception of De Ritis ratio, CTC-changed biochemical measurement in the blood plasma

reflecting the functional activity of hepatocytes (Figure).

The administration of Essentiale H and hypoxen in animals intoxicated with CTC normalized TAA and the activity of antioxidant defense enzymes (SOD and catalase) and corrected the concentrations of MDA and SM_{ON} (Figure).

The use of CLAH proteins in CTC-intoxicated animals normalized the level of AHP, the activity of SOD and catalase, TAA, corrected MDA and SM_{ON} concentrations (Figure).

Simultaneous introduction of CLAH proteins, Essentiale H and hypoxen to animals with acute toxic liver damage, in comparison with the previous groups of experimental animals, additionally normalizes the concentration of MDA and SM_{ON} in blood plasma (Figure).

The administration of pharmacological agents to the animals with ATLD normalizes the hemoglobin content in erythrocytes and brings the activity of SOD and the sorption capacity of cells closer to the normal parameters (Figure).

The introduction of CLAH proteins also normalizes the level of hemoglobin and corrects the other studied parameters of metabolic status in peripheral blood erythrocytes (Figure).

Combined administration of Essentiale N, hypoxen and CLAH proteins, in comparison with the previous groups of experimental animals, normalizes the concentration of MDA, SOD activity, sorption capacity of erythrocytes and their count in the blood, corrects the level of AHP to a greater degree towards the norm (Figure).

The introduction of Essentiale H and hypoxen to CTC intoxicated animals corrects the indices of phagocytic activity (PI, PN, EIA), NBT-ind. o/z and NBT-test of peripheral blood neutrophils and corrects the other studied parameters of oxygen-dependent metabolic activity in peripheral blood neutrophils (Figure).

The use of CLAH both separately and in combination with Essential H and hypoxen resulted in normalization or additional correction of the studied indices of functional and metabolic activity in peripheral blood neutrophils except for NBT-sp. and NBT-ind. n/z (Figure).

In animals that received Essentiale H and hypoxen under ATLD conditions, histological examination shows focal changes in the liver tissue in the form of large and small-drop adipose degeneration of hepatocytes, single small areas of necrosis with a weak inflammatory reaction.

Morphologically, in animals with ATLD, which received CLAH proteins, Essentiale H and hypoxen, the histoarchitecture of the liver tissue is preserved, the beam structure of the lobules is clear, nuclei are moderately basophilic in hepatocytes, the cytoplasm is oxyphilic, only small foci with granular dystrophy of the hepatocytes occur by places.

During morphological study of the liver tissue in groups of animals with acute toxic liver damage and intoxicated animals that received the proteins of CLFB, CLXH, CLAH, CLAH in combination with essentiale and hypoxen, in addition to generally accepted histological methods of research, to objectively verify and evaluate changes severity and peculiarities occurring in the liver parenchyma, morphometric methods of investigation were performed which were to determine the parameters characterizing the degree and peculiarities of ongoing processes and enabling to evaluate regenerative potential of the liver parenchyma.

In animals with acute toxic liver damage with CTC, in comparison with intact rats, changes in the values of morphometric indices are represented by a significant decrease in PD by 1.4 times, FCM by 2.1 times, NM by 2.2, BCMI by 2 times and an increase in MMI by 2.5 times (Table 4). These changes in indices are characterized by the development of congestive phenomena (plethora, cholestasis) in the liver tissue, an increase in the current (relatively short-term) intensity of hepatocyte functioning, their pronounced degenerative changes in the form of dystrophy and necrosis, an increase in the ongoing intensity of reparative processes (due to "binuclear reserves consumption") and a decrease in the depth of hepatic tissue reparative reserves.

Table 4

Morphometric parameters of the hepatic tissue status in animals that received proteins of the CLFB, CLXH, CLAH and CLAH in combination with essentiale H and hypoxen under the conditions of acute CTC poisoning (M ± m)

Item No	Indices / groups	PD	FCM x10^5	NM x10^5	BCMI x10^3	MMI
1.	Intact animals	0.53±0.03	10.54±0.8	11.73±0.9	2.27±0.1	53.8±2.9
2.	CTC	0.39±0.02^{*1}	4.96±0.3^{*1}	5.35±0.4^{*1}	1.16±0.08^{*1}	133.5±8.9^{*1}
3.	CTC + CLFB proteins	0.44±0.02$^{*1.2}$	5.88±0.4$^{*1.2}$	6.40±0.4$^{*1.2}$	1.48±0.08$^{*1.2}$	128.9±9.2^{*1}
4.	CTC + CLXH proteins	0.45±0.02$^{*1.2}$	6.41±0.5$^{*1.2}$	6.98±0.5$^{*1.2}$	1.51±0.08$^{*1.2}$	110.0±9.3$^{*1.2}$
5.	CTC + CLAH proteins	0.50±0.02^{*2-4}	9.05±0.6^{*2-4}	10.10±0.9^{*2-4}	2.22±0.09^{*2-4}	64.7±4.2^{*2-4}
6.	CTC + CLAH proteins + essentiale + hypoxen	0.51±0.03^{*2-4}	9.52±0.6^{*2-4}	10.70±0.8^{*2-4}	2.4±0.1^{*2-4}	59.0±3.9^{*2-4}

Note. The asterisk marked significant differences of average arithmetical ($p < 0.05$); figures close to the star are in relation to that of a group of these differences.

The introduction of CLFB and CLXH proteins into the animals under the conditions of acute carbon tetrachloride intoxication resulted in a positive dynamics of morphometric indices compared to animals without correction with proteins, however the absolute values of PD, FCM, NM, BCMI and MMI were significantly different from those in intact animals, that indicates the presence of congestive phenomena and degenerative changes in the liver tissue, intensive "binuclear reserves consumption" and a decrease in the depth of reparative reserves.

The administration of CLAH proteins and CLAH proteins in combination with essentiale and hypoxen in acute toxic hepatopathy manifested by insignificant changes in morphometric parameters, whose values did not differ with assurance from the group of intact animals. This bears evidence to high invariance of the histological structure, the absence of morphologically significant alternative changes and disturbances in reparative capabilities of the liver tissue in the animals of these groups.

Conclusion

In ATLD caused by carbon tetrachloride intoxication, the main biochemical syndromes of the liver cells damage, an increase in the phagocytic and oxygen-dependent activity of peripheral blood neutrophils, oxidative stress development, and the disturbance in erythrocytes metabolic activity have been revealed. In these conditions the use of culture liquid of fibroblasts in the presence of carbon tetrachloride intoxication partially normalizes and corrects metabolic disorders at the systemic and local levels. Administration of culture liquid of allogeneic hepatocytes in case of carbon tetrachloride poisoning normalizes and corrects metabolic disorders at the systemic and local levels more effectively, in comparison with the administration of culture liquid of xenogeneic hepatocytes.

In integral estimation of disturbed parameters amount under various experimental conditions with the determination of these disorders pronouncement by their degree, it was established that the use of pharmacological agents (Essentiale H and hypoxen) and culture liquid of allogeneic hepatocytes enabled to correct and normalize the immune and metabolic status disorders at the systemic level, whereas their concomitant use normalizes the studied indices practically entirely.

The development of biochemical syndromes of the liver tissue damage, the

activation of metabolic and functional activity of polymorphonuclear leukocytes, and the activation of lipid peroxidation in the blood plasma have been revealed in CTC intoxication. The administration of hepatocytes culture liquid of intact rats in combination with pharmacological preparations (Essentiale Forte H and Hypoxen) to allogeneic recipients intoxicated with CTC corrects systemic metabolic disorders arising from the action of hepatotrophic poison more efficiently, in comparison with their separate use.

Conflicts of Interest

The authors have no conflict of interest to declare.

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