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Research Article

Alterations in The Functional and Structural Properties of Erythrocytes Due to Alcohol Intoxication; Correction of Disorders

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Abstract. Alcohol abuse is among the leading reasons for disability and accounts for at least 5% of the global health burden. This study aims to define changes in the functional and structural properties of erythrocytes in chronic ethanol intoxication and to design methods for correction of the identified disorders. The experiments has been conducted on 93 healthy sexually mature and newborn Wistar rats. Acute and chronic alcohol intoxication has been designed by forced intragastric administration of 20% ethanol solution at a dose of 3 ml/kg (2.92 g/kg) on a daily basis for 5, 30 and 60 days. From the newborn animals we obtained allogeneic hepatocytes and their cultural fluid, from which proteins were isolated. The proteins of allogeneic hepatocyte and the combined administration of immunomodulatory, hepatoprotective and antioxidant agents were used to correct disorders in chronic intoxication. The membranes of circulating erythrocytes were obtained by the method of Dodge; electrophoretic separation of membrane proteins was carried out according to the method of Laemli. The lipid content in the erythrocyte membrane was determined by liquid thin-layer chromatography. It has been elucidated that in presence of chronic alcohol intoxication there is a change in the content of the membrane proteins in circulating erythrocytes, which are responsible for flexibility and shaping, intrinsic cellular metabolism, stabilization and structure formation, and a disruption in the lipids representativeness and ratio that form the basis for the lipid framework of the plasmalemma and play the major role in arrangement of protein macromolecules and the normal metabolism of red blood cells as well. When analyzing the corrective activity of the proteins of allogeneic hepatocytes and the combination Glutoxim + Mexidol + Heptral, we have established their high efficiency towards the disorders in the functional and structural properties of circulating erythrocytes caused by chronic alcohol intoxication.

Keywords: Alcohol Intoxication, Disorders, Functional and Structural Properties, Erythrocytes.

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1. INTRODUCTION

Alcohol abuse is one of the main causative factors of chronic diseases in the world. Within the past two decades, alcohol consumption on a per capita basis has increased by 1.5 times and reached 18 liters of absolute alcohol (pure alcohol), which is twice as high as the level deemed by the World Health Organization to be particularly dangerous for human health. Based on expert estimates, the number of people consuming such amount of alcohol is 3-5% of the total population[1,2]. The brain, liver and organs of the gastrointestinal tract are seriously affected by ethanol and its substitutes. The pancreas and liver are most sensitive to the alcohol effects; it is one of the leading etiological factors in the pathology of the pancreatic and biliary system [3,4]. The disorders in immune homeostasis, oxidative stress, and endothelial dysfunction are known to underlie the pathogenesis of the cardiovascular pathology, diseases of the lungs, the excretory and reproductive systems, gastrointestinal tract, liver, and pancreas[2,5]. The disorders leading to endothelial dysfunction are based on changes in the production of various biologically active compounds synthesized by vascular endothelial cells, among those the reactive oxygen species are of a greater importance, the excess production of which or a decrease in antioxidant defense factors results in the development of oxidative stress, activation of lipid peroxidation (LPO), oxygen-dependent activity of neutrophils, inhibition of endothelium-dependent vasodilation, increased synthesis of adhesive molecules, increased platelet aggregation and thrombosis. LPO end products, including unsaturated aldehydes and other metabolites, have strong cytotoxic, immunosuppressive, and mutagenic properties[3,6]. Within the past few years many authors have established in their studies the apparent role of erythrocytes in regulation of various metabolic processes, not only in normal conditions but, first of all, in pathological conditions[7]. At the same time, there are practically no data concerning the impact of alcohol intoxication on the structural components of the erythrocyte membrane. Red blood cells possess an enormous functional capacity. They perform O_2 and CO_2 transport by hemoglobin, the transfer due to sorption on the surface structure of the membrane or being presented to the bilipid matrix of amino acids, neurotransmitters, hormones, the immune system cytokines, lipids, medical preparations, the regulation of acid-base balance, hydro-electrolytic balance, microrheological state of blood. In recent years, there has been revealed a significant role of erythrocytes in the regulation of immune homeostasis both in the normal conditions and in various types of pathology, including the of hepato-pancreato-biliary system diseases[5,8,9]. At the same time, the literature data, concerning the changes in intrinsic cellular metabolism, structural components of the erythrocyte membrane and methods for their correction in acute and chronic ethanol effects, are rather insufficient. Considering the fact that alcoholic beverages are widely spread and available and that excessive ethanol or alcohol substitutes consumption is the major reason resulting in the growth of terminal stages of the liver diseases, it seems highly substantial to elucidate the influence mechanisms of hepatocytes, the liver and bone marrow stem cells, and their metabolic by-products in the liver pathology, involving that of alcoholic origin[10]. Hence, when analyzing scientific publications, it becomes obvious that there is a shortage of experimental information regarding the effect of cellular technologies in presence of alcohol intoxication. The present study mainly tries to define the alterations in the functional and structural properties of erythrocytes in acute and chronic ethanol intoxication and to design the methods for correction of the identified disorders.

2. MATERIALS AND METHODS

The experiments has been conducted on 93 healthy sexually mature Wistar rats, weighing 150-200 g, and newborn Wistar rats in the morning hours from 8 am to 12 pm. Keeping and killing of animals have been performed and approved according to:

1. Order of the Ministry of Health of the Russian Federation No 199n dated April 1, 2016 "On approval of the regulations for good laboratory practice";

"GOST 33216-2014. Interstate standard. Guidelines for accommodation and care of animals. Species-specific provisions for laboratory rodents and rabbits (enacted by the Order No 1733 of the Federal Agency on Technical Regulating and Metrology, dated 09.11.2015);Chronic and acute alcohol intoxication has been designed by forced intragastric administration of 20% ethanol solution at a dose of 3 ml/kg (2.92 g/kg) on a daily basis, 30 days (CAI-30) or 60 days (CAI-60)[9,11]. Killing of the experimental; animals was carried out in 24 hour following the last intoxication. To morphologically confirm the growth of simulated pathological procedures and perform a multiple evaluation of the preparations efficiency, a histological examination of the pancreas and liver has been carried out. For this purpose, the pieces from the above mentioned organs were fixed in 10% neutral formalin per 0.1 M phosphate buffer pH=7.2 and embedded in paraffin. The paraffin sections 7–10 μ m thick were stained with Regaud's hematoxylin and eosin. Preparation of allogeneic hepatocytes, their culture fluids, the proteins of culture fluid. Isolation of allogeneic hepatocytes (AH) was carried out from 5-6-day-old animals. Culture fluid of allogeneic hepatocytes was obtained by the method designed in our laboratory [7,12]. In order to acquire hepatocytic proteins from 50-100 ml of culture fluid, they have been precipitated in similar volumes along with 10% tritactic acid for thirty min; the precipitate has been separated through centrifugation for nearly thirty min at 3000 g. Following the supernatant has been separated, the sediment has been diluted with alkalized solution of 0.9% sodium chloride and dialyzed in phosphate-buffered saline with pH 7.2-7.4 for roughly eighteen hs. The dialyzed protein solution then has been clarified through centrifugation at 3000 g for 30 min and fractionated on a ULTROGEL AcA44 column. Protein from the column was brought in t2wo peaks: the initial one in the free volume having proteins of above 130 kDa; the next one, the leading peak, which has been entrapped in the gel, consisted of proteins under 130 kDa. After determining the concentration of protein in the peaks, the acquired fractions have been filtered by 0.2-μ m sterilizing membranes, dispensed into sterile vials, with one mg of protein per vial, and lyophilized on a VIRTIS freeze dryer. The acquired proteins of allogeneic hepatocytes (PAH) with MW under 130 kDa have been introduced 5 times intraperitoneally at the rate of 5 mg/kg of protein from the 55th day of intoxication with ethanol. Intoxication with ethanol at the doses and the frequency of their administration used 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 intoxication with ethanol, pharmacological preparations, or the proteins of culture fluid of allogeneic hepatocytes.

The methods, dosage, and pharmacological preparations' administration frequency are provided in Table 1. Determination of preparation dosages for administration to the experimental animals has been conducted through the factor of dose conversion (mg/kg per mg/m2) for a rat relying upon the weight of body or empirically, on the basis of LD_{50}.

Table 1. Methods, Dosages, and Preparation Administration Frequency to Animals with Dire Ethanol Intoxication

Preparations	Introduction means	One dose, mg/kg	Injection scheme	
			Number of injections (introductions)	Interval between injections (introductions), h
Glutoxim	intramuscularly	20	5	24
Mexidol	intraperitoneally	50	14	24
Heptral	intraperitoneally	760	5	24

Killing of rats was carried out in 24 hours after the last introduction of ethanol, preparations or PAH. Sampling of blood from the animals has been performed under anesthesia by intracardiac puncture. Erythrocytes and Plasma have been acquired from heparinized blood by centrifugation for five minutes at 400 g. *Study of the functional and structural properties of circulating erythrocytes.* When the plasma was separated, the erythrocyte mass has been put in twenty ml of ten mM Na-phosphate buffer (pH=7.4) having 0.90% sodium chloride and 3.0% dextran T-500 for 30 min at 37°C. Following that centrifugation the supernatant has been eliminated by aspiration, and the erythrocyte mass has been further purified on a chromatographic column through HBS-cellulose, the erythrocytes sorption capacity (SCE) and the sorption capacity of their glycocalyx (SCG) have been calculated [13,14]. Erythrocyte membranes were obtained by the method of Dodge [2,10], electrophoretic separation of membrane proteins was carried out in the presence of sodium dodecyl sulfate in vertical polyacrylamide gel[7,9]. For the linear preparation of the sample analyzed, 50 µl of erythrocyte membranes were taken for electrophoresis. Electrophoregrams were stained with Coomassie R-250[5,12,15]. Densitometry of one-dimensional electrophoregrams was carried out on a laser densitometer "'Ultroscan XL". Identification and calculation of protein fractions according to Steck-Fairbanks classification was carried out using the program OneDscan. The quantitative content of protein fractions was calculated using the obtained area of the required protein, marker protein and the known mass of the marker protein of human serum albumin. The protein concentration in the fractions was calculated in accordance with the established mass (µg) and expressed in micrograms per 1 µL of the total membrane protein, or mg%. The lipid content in the erythrocyte membrane was determined by the method of liquid thin layer chromatography[2,4,7]. In order to develop the chromatograms, the plates were treated with 5% alcohol solution of phosphomolybdic acid, followed by heating in a thermostat at 100°C for 5 min until there appeared blue spots of lipids. Identification of lipid fractions was carried out using the standards of "Sigma" company (USA). The obtained chromatograms were analyzed by means of the densitometer method using OneDscan program. The lipids count (mg/dL) was determined by the calibration curves of standard solutions. The severity of lipid peroxidation (LPO) procedures has been evaluated through the contents of malondialdehyde (MDA) and acyl hydroperoxides (AHP) in erythrocytes, utilizing a TBK-Agat kit (Agat-Med, Russia) when utilizing an Apel-330 spectrophotometer (Japan) at wavelengths of 570 nm and 535 nm. Using the approach of direct/competitive enzyme-linked immunosorbent assay (ELISA) with detecting reaction products at wavelengths ranging from 405 to 630 nm, the state of antioxidant system has been evaluated utilizing the kits to analyze the action of superoxide dismutase (SOD) generated by Bender Medsystems (Austria) and to evaluate the action of catalase generated by Cayman Chemical (USA). The degree of stable nitric oxide metabolites (SMON) has been defined utilizing an ELISA kit from R&D (England). All the ELISA outcomes were registered employing a Sunrise microplate photometer (Tecan, Austria).

3. STATITICAL ANALYSIS

Statistical process of the outcomes has been performed through defining the median (Me) and 25th and 75th percentiles employing the software package Statistica 8. The importance of differences has been evaluated by Mann-Whitney U test. The differences were deemed significant at $p<0.05$. A disruption degree of the laboratory factors (1) and alterations in the laboratory factors (2) under the effect of the pharmacological agents have been determined utilizing specific formulas[1,4,8].

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

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

4. RESULTS

In animals with five-fold introduction of ethanol there were no changes found in the proteins content of erythrocyte membrane. The introduction of ethanol for 30 days reduced the level of α - and β -spectrin, actin in the erythrocyte membrane and increased tropomyosin, while the content of the other membrane proteins studied remained normal. The introduction of ethanol for 60 days, in comparison with a 30-day intoxication, additionally reduced the representativeness of ankyrin and actin, increased the content of pallidin and, even to a greater extent, tropomyosin, while the content of anion-transport protein (ATP), band 4.1 and 4.5 protein, dematin, glyceraldehyde 3-phosphate dehydrogenase (G-3-PD) and Glutathione-S-transferase (G-S-T) were normal. A short-term intoxication with ethanol reduced the content of phosphatidylcholine (PC), phosphatidyl inositol (PI), sphingomyelin (SM), increased the content of lysophospatidylcholine (LPC), cholesterol (C), triacylglycerols (TAG), the amount of mono- and diacylglycerols (MAG, DAG) and non-esterified fatty acids (NEFA) in the erythrocyte membrane, with a normal content of phosphatidylethanolamine (PE), phosphatidylserine (PS), the amount of LPC, PC, PE, PS and PI (glycerophospholipids - GPL), the amount of GPL and SM (phospholipids - PL) and cholesterol esters (CE). The ethanol introduction for a whole month, in comparison with a short-term intoxication, further reduced the representativeness of PE, GPL and PL, to a greater extent reduced the level of PI, SM, and increased LPC and C. Intoxication with ethanol for 60 days, in comparison with a 30-day in-take, additionally reduced the representativeness of PC, PI, SM and increased the content of LPC, DAG + MAG, and NEFA (tab. 2).

Table 2. Lipid Content in the Erythrocyte Membrane in Acute and Chronic Ethanol Intoxication (Me (k25%; k75%)

Indicators	1	2	3	4
	Control	Introduction of ethanol		
		5 days	30 days	60 days
PC	24.0(15.7; 32.4)	22.6(20.1;23.0)*1	22.8(20.);23.2)*1	21.2(19.6; 23.5)*1-3
LPC	3.9(3.2; 4.6)	4.2(3.9;5.7)*1	5.0(4.3;6.2)*1,2	5.6(4.9; 6.9)*1-3
PE	24.6(23.6; 25.8)	24.1(23.1; 26.0)	23.0(22.1;24.8)*1,2	22.0(20.3; 24.5)*1,2
PS	19.7(18.8; 23.0)	18.8(17.2;24.1)	19.0(17.5;23.9)	19.2(18.1; 21.1)
PI	4.6(4.1; 4.9)	4.4(4.0;5.1)*1	4.1(3.8;4.6)*1,2	3.9(3.1; 4.7)*1-3
GPL	76.6(75.2; 77.9)	74.1(73.7;77.8)	73.9(72.1;74.7)*1	72.5(69.1; 74.8)*1
SM	12.2(11.5; 13.4)	11.1(10.2;12.7)*1	10.2(9.2;11.4)*1,2	8.9(7.2; 9.5)*1-3
PL	88.6(86.3; 90.0)	86.2(85.1;90.2)	84.1(82.7;91.3)*1	82.9(74.3; 89.2)*1
C	44.8(39.2; 52.8)	47.8(45.4;50.3)*1	52.4(51.0;55.8)*1,2	54.9(51.3; 57.1)*1,2
CE	40.0(37.7; 43.1)	39.8(38.2;44.6)	41.2(38.4;46.5)	39.3(34.5; 47.3)
TAG	14.5(11.8; 18.3)	16.1(14.4;19.6)*1	15.9(14.9;19.1)*1	16.2(13.1; 19.0)*1
DAG+MAG	9.6(8.4; 10.8)	10.5(9.2;12.5)*1	10.1(9.4;12.3)*1	11.4(8.4; 13.1)*1-3
NEFA	2.9(1.8; 4.1)	3.1(2.9;4.4)1*1	3.2(2.8;4.3)*1	3.5(2.9; 4.5)*1-3

Note: Here and in the tables 2, 3 and 4, an asterisk (*) marks significant differences in medians (p<0.05), percentiles 25% and 75% are indicated in brackets

When studying the ratio of membrane lipids fractions, it was determined that a short-term intake of ethanol increases the ratio of C+CE/PL, a 30-day introduction increases the ratio of LPC/PC, even to a greater extent C+CE/PL and reduces the ratio of SM/PC. Intoxication with ethanol for 60 days reduces the ratio of SM/GPL, PC/PS, PC+PE/PS+PI, and to a greater extend increases the ratio of LPC/PC. When assessing the indicators of erythrocyte metabolism, it was found out that there was observed a decrease in their total count, the SOD activity and sorption parameters due to a 5-day ethanol intake. In CAI-30, there was revealed a decrease in the number of erythrocytes, an increase in the AHP activity, and a reduction in the antioxidant defense factors (SOD and the catalase activity). In a 60-day intake of ethanol, in comparison with CAI-30, LPO indicators (AHP, MDA) were increased and the activity of antioxidant defense enzymes (SOD, catalase) was reduced. It was essential to determine a multidirectional change in the sorption capacities of erythrocytes: in a short-term intoxication with ethanol SCE and SCG were reduced, while in a long-term intoxication these indicators, on the contrary, were increased. Taking into account the literature data and our previous experience concerning the use of pharmacological preparations with immunomodulatory, antioxidant and membrane-protective activity in experimental and clinical practice[1,3,16], we choose the same strategy for pharmacological correction of the disorders identified in CAI-60 with the most altered parameters of the structural and functional properties of erythrocytes. Besides, the pharmacological correction of disorders in the functional and structural properties of erythrocytes due to chronic alcohol intoxication with the help of proteins of allogeneic hepatocytes was studied. A 60-day ethanol intoxication reduced the content of α -β -spectrin, ankyrin and actin in the erythrocyte membrane of the experimental animals, with the simultaneous increase in the content of pallidin and tropomyosin. In this context the combination of glutoxim, mexidol and heptral normalizes the representativeness of pallidin and brings the content of α -spectrin, ankyrin and tropomyosin closer to the norm. Proteins of allogeneic hepatocytes had similar properties concerning the correction of the membrane proteins representativeness in circulating erythrocytes of animals with CAI-60 (Tab. 3).

Table 3. Representativeness of Membrane Proteins in Erythrocytes due to Continuous Ethanol Intoxication; the Disorders Correction by the Proteins of Allogeneic Hepatocytes (Me (k25%; k75%)

Indicators	1	2	3	4
	Control	60-day intoxication with ethanol and introduction:		
		without any preparation introduced	Glutoxim, mexidol, heptral	Proteins of allogeneic hepatocytes
α -spectrin	108.3(96.3; 117.9)	90.5(86.1; 95.9)*1	101,5(96.4; 04.1)*1,2	99.7(93.3; 102.1)*1,2
β -spectrin	103.1(100.4;111.4)	92.5(85.6;95.8)*1	102.4(94.5;106.7)*2	105.2(98.4; 108.4)*2
Ankyrin	89.0(85.7; 92.3)	74.2(70.7;78.0)*1	81.4(76.4; 87.0)*1,2	79.9(69.5; 88.1)*1,2
ATP	171.6(166.7;180.9)	169.8(156.7;174.4)	170.4(164.2;178.3)	167.4(163.2; 181.5)
4.1	70.4(67.9; 88.3)	71.2(68.5;75.5)	73.1(64.4; 79.7)	69.7(63.8; 78.7)
Pallidin	92.1(87.6; 99.3)	99.7(93.1;107.6)*1	91.2(86.6; 101,0)*2	90.4(85.3; 100.8)*2
4.5	79.8(76.3; 88.8)	78.0(75.4; 81.0)	77.9(70.3; 84.1)	81.2(77.4; 85.6)
Dematin	91.2(87.2; 98.9)	90.1(86.4;102.8)	89.3(83.7; 94.5)	87.5(81.4; 90.0)
Actin	88.1(83.7; 94.5)	68.3(63.4; 74.3)*1	80.2(71.3; 85.7)*1,2	84.5(82.7; 91.6)*1,2
G-3-PD	54.3(45.9; 64.1)	56.2(49.1; 62.3)	55.2(50.0; 61.6)	52.1(47.1; 60.5)
Tropomyosin	63,7(58.1; 70.7)	84,6(75.6; 89.6) *1	75.1(68.6; 79.5)*1,2	77.0(63.4; 87.5)*1,2
G-S-T	61.2(55.9; 74.3)	62.8(53.5; 70.1)	63.5(59.4; 67.8)	64.1(58.6; 71.4)

In presence of 60-day ethanol administration, the level of PC, PE, PI, GPL, SM, PL, the ratio of SM/PC, SM/GPL, PC+ SM/PE+PS+PI decreases in erythrocyte membrane. At the same time, there was revealed an increase in the content of LPC, C TAG, the sum of MAG and DAG, NEFA, the ratio of LPC/PC and C+CE/PL (Table 4). The use of the pharmacolog ical composition normalizes the representativeness of PC, LPC, PI, TAG, DAG + MAG, NEFA, the ratio LPC/ PC and PC + SM/ PE + PS + PI, corrects, but not to the level of the healthy animals, the content of PE, GPL, SM, PL, C and C+CE/PL ratio. The use of PAH in presence of ethanol intoxication additionally normalizes the representativeness of PE, GPL, and PL in comparison with the pharmacological preparations (Table 4).

Table 4. Representativeness of Lipids in Erythrocyte Membrane in Prolonged Ethanol Intoxication; the Disorders Correction by Proteins of Allogeneic Hepatocytes (Me (k25%; k75%)

Indicators	1	2	3	4
	Control	60-day intoxication with ethanol and introduction:		
		Without any preparation introduced	Glutoxim, mexidol, heptral	Proteins of allogeneic hepatocytes
PC	24.0(15.7; 32.4)	21.2(19.6; 23.5)*[1]	24.6(23.4; 25.8)*[2]	25.4(23.2; 28.1)*[2]
LPC	3.9(3.2; 4.6)	5.6(4.9; 6.9)*[1]	4.0(3.3; 4.7)*[2]	4.1 (3.3; 4.6)*[2]
PE	24.6(23.6; 25.8)	22.0(20.3; 24.5)*[1]	23.5(22.7; 24.8)*[1,2]	25.5(23.6; 28.1)*[2]
PS	19.7(18.8; 23.0)	19.2(18.1; 21.1)	19.4(18.5; 21.2)	20.1(18.2; 21.8)
PI	4.6(4.1; 4.9)	3.9(3.1; 4.7)*[1]	4.5(3.9; 5.0)*[2]	4.7(4.2; 5.1))*[2]
GPL	76.6(75.2; 77.9)	72.5(69.1; 74.8) *[1]	74.8(73.1; 76.2)*[1,2]	79.8(74.6; 85.2)*[2,3]
SM	12.2(11.5; 13.4)	8.9(7.2; 9.5)*[1]	10.6(9.2;12.0)*[1,2]	10.8(9.1;12.8)*[1,2]
PL	88.6(86.3; 90.0)	82.9(74.3; 89.2)*[1]	86.4(84.9; 89.8)*[1,2]	90.6(87.1; 93.4)*[2,3]
C	44.8(39.2; 52.8)	54.9(51.3; 57.1)*[1]	48.9(46.9; 50.3)*[1,2]	49.5(43.2; 51.4)*[1,2]
CE	40.0(37.7; 43.1)	39.3(34.5; 47.3)	42.0(38.2; 47.7)	41.6(39.4; 49.0)
TAG	14.5(11.8; 18.3)	16.2(13.05; 19.0)*[1]	14.1(12.9; 16.7)*[2]	15.0(12.5; 17.8) *[2]
DAG+MAG	9.6(8.4; 10.8)	11.4(8.4; 13.1)*[1]	9.9(7.6; 11.4)*[2]	9.5(8.1; 12.0)*[2]
NEFA	2.9(1.8; 4.4)	3.5(2.9; 4.5)*[1]	2.8(1.9; 3.8)*[2]	2.7(1.7; 3.7)*[2]

When assessing the metabolic activity of circulating erythrocytes in CAI, in comparison with the control, an increase in LPO products (MDA, AHP), a decrease in antioxidant defense factors (a decrease in TAA and the activity of SOD and catalase enzymes) and sorption capacity of the red cell membrane (SCE, SCG) were revealed. The combined administration of the preparations "Glutoxim", "Mexidol" and "Heptral" normalizes TAA, the catalase activity and sorption capacity of erythrocytes (SCE and SCG). The use of PAH, in comparison with the pharmacological preparations, corrected to a greater extent, but not to the level of the control animals, the state of LPO (MDA, AHP content) and normalized antioxidant defense factors - TAA and the activity of SOD enzyme. In presence of CAI, there was revealed the effectiveness of antioxidant, hepatoprotective and immunomodulatory agents and the proteins of allogeneic hepatocytes. It goes without saying, there is a need to use various statistical methods to compare the effectiveness of the schemes applied for the correction of the disorders that occur due to prolonged ethanol intoxication. In chronic long-term intoxication with ethanol, 29 (76.3) of the studied laboratory parameters characterizing the functional and structural properties of circulating erythrocytes are disrupted. The introduction of the pharmacological composition "Glutoxim", "Mexidol", "Heptral" normalized 51.7%, corrected 41.4% and left unchanged 6.9% of the parameters, respectively. The use of the proteins of allogeneic hepatocyte normalized 62.1%, corrected 31%, and left initially changed 6.9% of the studied laboratory parameters, respectively. To qualitatively compare the disrupted immune-metabolic parameters, they were analyzed by the level and dividing the severity of disruption by degrees [7-9]. In chronic long-term intoxication with ethanol, 29 (76.3%) of the studied laboratory parameters that characterize the functional and structural properties of circulating erythrocytes were disrupted, 69%, 20.7% and 10.3% of which turned out to be of the I, II and III degrees of disruption, respectively. The combined use of "Glutoxim", "Mexidol", "Heptral" reduced till 36.8% the parameters changed from the control, characterizing the functional and structural properties of erythrocytes, and as for the quality according to the degrees of disruption, 92.9% were disrupted in the I degree, 7.1% - in the II degree, but there was not revealed any disruption of the III degree. The introduction of PAH proved out to be more efficient, due to the fact that it reduced the changed from the control parameters, characterizing the functional and structural features of erythrocytes to 28.9%, and as for the quality, in accordance with the degree of disruption, 81.8% of parameters were disrupted in the I degree, 18.2% - in the II degree, but there was not any disruption revealed in the III, respectively.

5. DISCUSSION

According to the literature data, modern society is characterized by an increase in the number of people who abuse alcohol, psychotropic and narcotic agents, that results in the incidence rise of diseases associated with these pathological addictions. Alcohol abuse is known to cause hypovolemic disorders, changes in the protein composition of the blood, adversely affecting the organs of the digestive system, the nervous system and cardiotrophic functions. Metabolic shifts in neurocytes, cardiomyocytes, and liver cells cause functional impairment of the regulatory systems [11-13]. The framework of the erythrocyte membrane, like the membrane of any other cell, is formed by a double phospholipid layer with asymmetrically integrated proteins. The main structural features of the biological membrane of erythrocytes are determined by the properties of the lipid bilayer, but most of its specific functions are carried out by proteins, with a strict arrangement of the protein macromolecules, which are divided into integral and peripheral ones, the latter include a number of erythrocyte enzymes, and up to 80% of them are part of its cytoskeleton, which is an elastic two-dimensional network connected directly to the membrane through interaction with the polar groups of integral proteins and the polar lipid heads [4-8,17]. The revealed data indicate the significant changes due to a long-term intake of ethanol in the peripheral proteins responsible for the structure formation and stabilization of the erythrocyte membrane (α- and β-spectrin, ankyrin, pallidin), and the shaping and flexibility of the membrane (actin, tropomyosin) as well[5,6]. All the cells of animal origin, including erythrocytes, contain the outer and inner layers of PL in the membranes, and the distribution of their individual representatives in the membrane is asymmetric

to PC and SM - they are neutrally charged and contain choline, they are located mainly in the outer part of the membrane, when FE and PS - with the presence of an amino group in the nitrogenous base, and FI that does not contain nitrogen, are located, mainly, in its inner layer. Due to this, there takes place the interaction of regulatory and structural proteins, the membrane mechanical properties and the cell shape are maintained and there is a greater flow of the inner monolayer[7-11]. There were also the disorders in the content of the lipid spectrum: a decrease in the level of PC, PE, PI, the ratios SM, SM/PC, SM/PL, PC+SM/PE+PS+PI, the content of glycero- and phosphosphingolipid membranes (the sum of LPC, PC, PE, PS, PI and SM), constituting the skeleton of the cell membrane and arranging its protein and sphingoglycolide components, a change in phospholipids localized mainly on the outer surface of the cytoplasmic membrane towards phospholipids of its inner part (PC + SM/ PE + PS + PI) [12,13,18], an increase in the representativeness of LPC, TAG, DAG + MAG, NEFA, the LPC/PC ratio (the most powerful factor in modifying the properties of the lipid bilayer and integral membrane proteins that play a major role in the normal metabolism of erythrocytes), an increase in C content, the C + CE/PL ratio, disrupting the function of receptors and membrane enzymes, along with structural changes in proteins, which are mostly the enzymes, carriers and receptors, that undoubtedly leads to the disorders in intrinsic cellular metabolism and functional properties of circulating red blood cells[6,9]. Endoglobular increase in LPO processes and the content of stable nitric oxide metabolites, a decrease in the activity of antioxidant defense enzymes [8,13] results in the serious disorders in the functional properties of peripheral blood erythrocytes, which is confirmed in our studies by pronounced disruptions in the sorption properties of the red blood cells membrane. PC has a high exchange activity, it is of great importance for the membrane permeability, it affects the C metabolism, and plays a special regulatory role in LPO processes in the normal conditions and in case of some pathological conditions development. The normal SM and PC, SM and GPL ratios determine both the osmotic and hemolytic stability of erythrocytes, and also their thermal stability and continuous circulation in the bloodstream. PC and PE are specific activators of some membrane-bound enzymes, have an effect on stem cells, activate the monocyte system, and retain the antioxidant activity of the membrane lipids. PS plays an extremely important role in the process of erythrocytes apoptosis, which is one of the ways to renew blood corpuscles. The membrane PI increases the ability to control the transport and information transmission into the cell in the form of humoral signals, affects the physical-chemical properties of the membrane, its viscosity and ability to bind calcium ions, in particular, which, in their turn, underlie the changes in the membrane functions, and the products of its catabolism act as the second messengers. The C presence in the bilayer contributes to its induration, reduces the permeability for small water-soluble molecules and water, increases elasticity and mechanical strength, and changes the activity of membrane proteins. Influenced by C, the membrane can change its shape in response to the force used to it, redistributing between the membrane monolayers. What is more, C is able to combine with PL in the erythrocyte membrane and deposit in the form of cholesterol clusters between its layers, disrupting the receptors and enzymes functioning. An increase in the content of C and CE can also contribute to a change in the shape of erythrocytes[8,11].

6. CONCLUSION

Hence, given the results obtained, it has been established that in CAI, not only the content of individual membrane fractions of lipids changes significantly, but also their relation to each other, that disrupts the structure formation of the cell membrane, changes in the arrangement of protein macromolecules and normal erythrocyte metabolism Furthermore, the results demonstrate that the most likely cause of the disorders in the protein-lipid spectrum of the erythrocyte membrane in toxic and ischemic liver damage is the intensification of free-radical processes, resulting in the increase of their sensitivity to peroxide processes An excessive activation of LPO processes is accompanied by significant changes in the composition, ratio and degree of oxidation of the membrane glycerophospholipids (PC and PE to a greater extent), phosphosphingolipids and glycosphingolipids, which, in the end, leads to the change in the physical-chemical properties of the lipid bilayer of cell membranes and a decrease in the activity of phospholipid-dependent enzymatic systems. In presence of excessive activation of free radical processes, in the first instance phospholipids containing polyunsaturated fatty acids naturally decrease. Moreover, selective defatting of the cytoplasmic membranes of erythrocytes causes an increase in the ratio between the content of cholesterol and phospholipids in the bilayer of cell membranes, which ultimately leads to erythrocytes dysfunction. Moreover, mature erythrocytes are not capable of synthesizing proteins and lipids; maintenance and change in their content and ratio is due to the erythrocytes micro-environment, namely the composition of blood plasma, that changes significantly in pathology, including CAI, which, in its turn, has a significant impact on the cell morphology, lipid-protein interactions in the erythrocyte membrane and the resulting activity of its enzyme systems. Summarizing the data obtained, it can be concluded that the combined introduction of agents with immunomodulatory, antioxidant and hepatoprotective activity, and also the administration of the proteins of culture fluid of allogeneic hepatocytes to the recipients in presence of CAI, limits the processes of free radical oxidation, contributing in such a way to the restoration of the functional and structural properties of peripheral blood erythrocytes.

7. AUTHORS' CONTRIBUTIONS STATEMENT

A.V. Kharchenko, E.S. Litvinova, N. A. Konoplya, and A.V. Serezh-kina equally designed, supervised and conducted the study. All authors discussed the results and commented on the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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