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RESEARCH ARTICLE

Features of lipid metabolism in membranes of rat cells at experimental traumatic brain injury on the background of chronic alcohol intoxication

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What is not yet known on the issue addressed in the submitted manuscript

Features of lipid metabolism in the membranes of erythrocytes and mitochondria of the cerebral cortex of rats with traumatic brain injury on the background of chronic alcohol intoxication.

The research hypothesis

Study of lipid metabolism in the membranes of erythrocytes and mitochondria of the cerebral cortex in experimental traumatic brain injury on the background of alcohol intoxication.

The novelty added by manuscript to the already published scientific literature

In experimental traumatic brain injury on the background of chronic alcohol intoxication significant and more pronounced changes in the content of total phospholipids, total cholesterol, their molar ratio in the membranes of erythrocytes and mitochondria of the cerebral cortex were revealed than in separately reproduced pathologies, as well as discoordination of the metabolism of individual fractions of phospholipids in membranes erythrocytes. The revealed pathophysiological features will make it possible to search for new remedy of pharmacological correction of targeted action.

Abstract

Introduction. Alcohol intoxication is often the cause of traumatic brain injury. The purpose of the work was to study the features of lipid metabolism in erythrocytes and mitochondria of the cerebral cortex cell membranes of rats in traumatic brain injury on the background of chronic alcohol intoxication.

Materials and methods. The studies were carried out on 140 male rats of the Wistar line weighing 170-250 g. Chronic alcohol intoxication was caused by using 15% ethanol as the only source of fluid for 20 days. Reproduction of traumatic brain injury was carried out by a shock model. Statistical analysis of data was conducted by using „Primer Biostatistics 6.0". The criteria of parametric statistics were used. The level of $p < 0.05$ was statistically significant.

Results. Chronic alcohol intoxication in rats and inducing of traumatic brain injury separately causes a significant change of total phospholipids and cholesterol content, and their molar ratio in the membranes of erythrocytes and mitochondria of the cerebral cortex. Particularly pronounced were the changes in studied objects with combined pathology, meaning, traumatic brain injury on the background of chronic alcohol intoxication.

Discoordination of lipid metabolism was unidirectional, manifested in a significant reduction in the content of total phospholipids, and increased total cholesterol, which led to a violation of the molar ratio of total cholesterol/phospholipids in the direction of increasing the coefficient, which is normally about 1.0.

In subsequent monitoring periods, there was a gradual restoration of individual fractions of phospholipids, but on the 30th day of the study, it did not reach the control values. That means that discoordination of the metabolism of individual phospholipid fractions was so pronounced, that even on 30th day of arbitrary reproduction did not reach the initial values, which again emphasizes the severity of the morphofunctional state of red blood cells membranes' disturbance.

Conclusions. The revealed features of lipid metabolism in the membranes of erythrocytes and mitochondria of the cerebral cortex in traumatic brain injury on the background of chronic alcohol intoxication are an important component for understanding the ongoing pathophysiological processes and searching for new effective drugs with targeted action.

Key words: membranes, erythrocyte, mitochondria of the cerebral cortex, alcohol intoxication, traumatic brain injury, lipids, phospholipids.

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Introduction

Chronic alcoholic intoxication for most countries of the world is a topical social and economic problem, the global manifestations of which are the appearance of a number of serious diseases and their complications, persistent loss of function and early disability. It is difficult to find organs and tissues in human body that would not be involved in damaged in chronic alcohol poisoning, in case of excessive alcohol abuse. The cardiovascular system, the pancreas, muscles, the immune system, and others are sensitive to an alcohol abuse damage. Especially high sensitivity to alcohol exhibits the liver, where its main metabolism occurs, with the formation of a highly toxic intermediate product, which is acetaldehyde [1]. There is no doubt that the most sensitive to the action of alcohol are central and peripheral nervous system, which results in systemic polyneuritis with functional impairment and pain manifestations and alcoholic encephalopathy [2].

Fundamentally in the multitropic damaging effect of alcohol is the initiation of a free radical chain of oxidation with the formation of partially oxidized products, which are aldehydes and ketones [3]. They cause morphofunctional changes in cellular membranes in the body, which consist of polymerizing effects on the protein component of the membranes with violation function of the phospholipid bilayer. Intensification of lipids peroxidation processes under the influence of toxic factors and, first of all, alcohol, is universal, nonspecific. Features of the structure and function of the central nervous system to a large extent due to the enormous variety of their lipid components, their properties, localization and metabolism [3]. This concerns both the content of total cholesterol (CE) and total phospholipids (PL), their molar ratio. The main structural unit of cellular lipids are PL, which represent a large family of phosphorus-containing natural lipids. Phospholipids form the main component of cell membranes and directly participate in the basic metabolic processes of the living cell. The most important function of PL is structural, since they form the phospholipid bilayer of all cell membranes. The composition of PL and their placement in membranes largely determines barrier properties of membranes, their permeability for various exo- and endogenous substances, ultimately their functional capacity [4]. Damage of the cell membranes, including neurons, consists of their destruction, which leads to a violation of the permeability for ions. In recent years established the undoubted role of erythrocytes in the regulation of various metabolic processes, both in normal and in the pathological conditions [5, 6]. It is also important that erythrocytes are the most accessible object for research, especially in clinical practice for assessing the patient's condition, the course of the disease and as a criterion for the effectiveness of the prescribed pharmacotherapy [7-9]. At the same time, practically there is no data about the influence of alcohol intoxication on the structural components of membranes of red blood cells. Often, alcohol intoxication is the cause of general injury and traumatic brain injury (TBI) is not the least. By itself, TBI causes a number of injuries both in the whole organism and in the brain. In the first place, this is an ischemic injury of cells that induces rigid hypoxia, which develops as a result of a violation of the morphofunctional state of cell membranes [9]. For normal functioning of the cell, lipids of the membranes must be in constant motion. Immobilization of lipids, which occurs as a result of TBI, alcohol intoxication, is associated with a qualitative rearrangement of the fatty acid pool and leads to a change in the lipid enzymes envelope and, consequently, to dysfunction of the cell [1, 8, 9]. In this regard, the maintenance of a stable structure and integrity of cell membranes is largely depends on the adaptive reorganization of the composition and the quantitative characterization of the cellular lipids bilayer [10]. Therefore, the studying of discoordination of lipid metabolism of cell membranes is a necessary step for the search and creation of a targeted action of drugs for the treatment of TBI on the background of chronic alcohol intoxication.

The purpose of the work – studying the features of lipid metabolism in membranes of erythrocytes and mitochondria of the cerebral cortex of rats at the traumatic brain injury on the background of alcohol intoxication.

Materials and methods of research

The research was conducted on 140 male rats of the Wistar line weighing 170-250 g at the age of 6 months from birth in the conditions of a chronic experiment in accordance with the requirements of the commission on bioethics of Odessa National Medical University. Animals were divided into four groups with 35 rats in each. In turn, each group were divided into 5 subgroups with 7 animals. The first subgroup served as control, the second – reproduced the experimental pathology, the third, fourth and fifth traced the restoration of the studied indicators in time intervals after 10, 20 and 30 days.

Chronic alcoholic intoxication, which was accompanied by the formation of alcohol addiction, caused by 20-days experiment in the „benefits of ethanol" test [11]. The animals were placed in individual boxes in which they had access to two drinking bowls: one with pure water and the second with 15 % ethanol. After measuring the volume of the drawn liquid, the coefficient of the alcohol preferences (K_n) was calculated: $K_n = 100\ \% \cdot V_{alcohol}/V_{general}$, where $V_{alcohol}$ is the volume of alcohol consumed, $V_{general}$ is the total volume of liquid drunk. The criterion for selecting animals for the subsequent experiment was the triad: drinking behavior; the preference for ethanol (with K_n not less than 50%) and the severity of neurological symptoms (by open field test and rotator test) [11]. The traumatic brain injury was reproduced by drawing a single blow to the crown of the head – the occipital part of the brain with a weight of 100 g, falling from a height of 80 cm, what is called the shock model [12].

In lipid extracts of erythrocytes the content of total CE and total PL in mmol/l were determined and also was calculated their molar ratio, which expressed by the coefficient of CE/PL. Blood was obtained from the caudal vein of rats. In parallel, in the lipid extracts of the membranes of the cerebral mitochondria, which were obtained by differential centrifugation, the content of total CE and total PL in mg/g were

determined and their molar ratio was calculated [13]. The isolation of membranes of erythrocytes and mitochondria were carried out by automatic pumping with registration and control on the spectrophotometer „Uvikor D-SH-2089 LKB", „Beckman" company with graphic registration of the peaks of the transition of environments and the measurement of their volumes. The control of the purity of the selection of membranes were performed by using a microscope. Lipid extracts were isolated from 1 ml of erythrocytes and 200 mg of cerebral cortex by method J. Folch et al. [13]. Fractionation of PL was done by the one dimensional ascending method of thin layer chromatography (TLC) [14]. The content of separate phospholipids: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), lysophosphatidylcholine (LPC), phosphatidylserine (PS), and sphingomyelin (SM) were evaluated by "combustion" of spots with 72 % chloric acid at 200°C until their complete discoloration with the following definition of lipid phosphorus [14]. The content of common PL was calculated based on the sum of individual fractions. As it is known from the scientific literature, the amount of inorganic phosphorus identified in PC, PE, PI, LPC, PS and SM is about 75-80% phosphorus from the remaining phospholipids and phosphate acids (Phosphatidic acid phosphatase (PAP)) [15]. Determination of inorganic phosphorus was carried out by the procedures developed by Chen's modification (2004), which was based on using ascorbic acid as a reducing agent. Measurement of protein in the membrane sample of erythrocytes were performed by using the Lowry method, which combines the biuret reaction and the reaction with Folin's reagent [16].

Statistical analysis of data was conducted by using „Primer Biostatistics 6.0". The criteria of parametric statistics were used. The level of $p < 0.05$ regarded as statistically significant.

Results of the research and discussion

Investigation of damaging effects mechanisms of various pathological factors, including exogenous chemical compounds, on the human and animal body is one of the priority tasks of medicine. Particularly relevant in this case is acquiring the definition of the role of structural and functional state of cell membranes, through which the resistance of the organism to the damaging effect and the restoration of the functioning of organs and systems are substantially mediated, including prescribed pharmacotherapy. The last decade was marked by an active study of the role of membrane mechanisms in the development of a number of diseases, maladaptive changes in the body [17]. It is also known that the implementation of various determinants was largely due to the influence on the phospholipid composition of membranes and the associated activity of marker enzymes. That is, cellular and subcellular membranes, in our case, the membranes of erythrocytes and mitochondria of the brain of rats, in the course of their conformational changes, play a barrier function and they are the first line of defense or targets of primary influence [18]. What is their protective function? A number of researches in our laboratory has proven that cellular or subcellular membranes form the framework of the corresponding structure, separating the contents of the internal environment from the external, thereby maintaining their morphofunctional ability. Maintaining of internal compartment state ensures the stability of the concentration and electrochemical gradients, membrane transport capabilities. On the quantitative and qualitative composition of total lipids, their ratio, the state of phospholipid fractions depends on the stability of the membrane basis, the functional capacity of the multienzyme complexes in the bilayer etc. [19]. On the other hand, it is definitely known that alcohol intoxication as traumatic brain injury discoordinate systemic mechanisms of self-regulation of membranes, which leads to metabolic disorders, acidosis, hypoxia, oxidative stress [20].

Proceeding from the above, the primary task was to study the dynamics of the content of total PL and total CE and their molar ratio in chronic alcohol intoxication and arbitrary recovery. These time intervals are important not only for understanding the period of disease formation, but also for approbation of the possibility, in the future, of medical correction. At the 20th day of alcohol intoxication, the most significant changes develop in the lipid profile of membranes of erythrocytes (table 1).

Table 1. *Dynamics of total cholesterol content, total phospholipids and their molar ratio in rats with chronic alcohol intoxication and arbitrary recovery (n=7).*

№ n/n	Experiment conditions	Statistical indicators	Erythrocyte membranes (mmol/l)			Mitochondria membranes of the cerebral cortex (mg/g)		
			Total CE	Total PL	Ratio CE/PL	Total CE	Total PL	Ratio CE/PL
1	Control	M±m	4.96±0.16	5.79±0.12	0.86±0.08	4.05±0.21	4.64±0.16	0.87±0.07
		%	100.0	100.0	100.0	100.0	100,0	100.0
2	Alcohol intoxication (20 days)	M±m	6.52±0.20	3.87±0.08	1.68±0.10	7.53±0.27	2.69±0.09	2.80±0.11
		%	131.4*	66.8*	195.3*	186.0*	58.0*	321.8*
3	10 days after alcoholization	M±m	7.56±0.22	3.17±0.09	2.38±0.13	7.01±0.19	3.14±0.11	2.23±0.14
		%	152.4*	54.7*	276.4*	173.1*	67.7*	256.3*
4	20 days after alcoholization	M±m	6.41±0.17	3.91±0.11	1.64±0.09	6.12±0.24	3.56±0.12	1.72±0.10
		%	129.2*	67.5*	190.7*	151.1*	76.7*	197.7*
5	30 days after alcoholization	M±m	5.20±0.10	5.11±0.18	1.02±0.06	5.24±0.15	3.97±0.19	1.32±0.06
		%	104.8*	88.2	118.6*	129.4*	85.6*	151.7*

Note: * - the significant differences between groups as relative to control
CE - cholesterol; PL - phospholipids

The content of total PL was reduced on a third part (from 5.79±0.12 in the control to 3.87±0.08 mmol/l, P < 0.05), and the content of total CE increased on a third part (from 4.96±0.16 in control to 6.52±0.20 mmol/l, P < 0.05). As a result, the molar ratio of CE/PL changed. It should be noted that in normal condition this ratio is about 1.0. In our study, on the 20th day of the development of alcohol intoxication, the ratio of CE/PL increased almost in 2 times (from 0.86±0.08 to 1.68±0.10, P < 0.05), indicating significant disproportion between total CE and total PL. At the parallel study of the composition of lipids of membranes of the mitochondria of the cerebral cortex, which is the most affected by alcohol intoxication, it was found that on the 20th day of alcohol abuse the content of total PL decreased by 2 times (from 4.64±0.16 in the control to 2.69±0.09 mg/g, P < 0,05), and the content of total CE increased by almost 2 times (from 4.05±0.21 in the control to 7.53±0.27 mg/g, P<0.05), table 1.

Accordingly, the ratio of CE/PL changed more than in 3 times (by 321.8%), indicating that the cerebral mitochondria membranes are more susceptible to the action of alcohol. Observation of the arbitrary restoration of the studied parameters in erythrocyte membranes during 30 days showed that the content of the total CE decreased gradually, on the 10th and 20th days of the study decreased and on 30th days reached the control values (5.20±0.10 and 4.96±0.16 mmol/l in the control, P > 0.05). The content of total PL, similarly to CE, gradually increased and for 30th day probably did not differ from the control (5.11±0.18 and 5.79±0.12 mmol/l in the control, P > 0.05). The ratio of CE/PL was also gradually aligned (0.86±0.08 in the control and 1.02±0.06 on the 30th day of the study). A similar, but less pronounced trend was observed in the membranes of the mitochondria of the cerebral cortex (table 1).

The results of the study indicate that the membranes of the cerebral mitochondria are not only more sensitive to the effects of alcohol, but the restoration of the lipid composition occur less clear and intense, and is more obvious in prolonged terms. At the 30th day of the study neither the content of total CE, nor total PL did not return to ascending values. The evidence of this is the ratio of CE/PL, which for 30 days remained 151.7% higher than in the control (1.32±0.06 at 0.87±0.07 in the control, P < 0 05),

The study of the dynamics of the content of total CE and total PL in the experimental traumatic brain injury in rats demonstrated the same tendency as in chronic alcohol intoxication, but the severity of these changes were much higher (table 2).

Table 2. *Dynamics of total cholesterol content, total phospholipids and their molar ratio in rats under traumatic brain injury and arbitrary recovery (n=7).*

№ n/n	Experiment conditions	Statistical indicators	Erythrocyte membranes (mmol/l)			Mitochondria membranes of the cerebral cortex (mg/g)		
			Total CE	Total PL	Ratio CE/PL	Total CE	Total PL	Ratio CE/PL
1	Control	M±m	4.32±0.15	5.11±0.11	0.84±0.05	3.98±0.09	4.55±0.11	0.87±0.03
		%	100.0	100.0	100.0	100.0	100.0	100.0
2	1 day after TBI	M±m	6.59±0.17	2.68±0.07	2.46±0.12	7.74±0.22	2.77±0.05	2.79±0.12
		%	152.5*	52.4*	292.8*	194.5*	60.9*	320.7*
3	10 days after TBI	M±m	7.03±0.13	2.85±0.06	2.47±0.11	8.06±0.24	2.65±0.06	3.04±0.13
		%	162.7*	55.8*	294.0*	202.5*	58.2*	349.4*
4	20 days after TBI	M±m	6.28±0.11	3.15±0.09	1.99±0.09	6.43±0.19	2.26±0.09	2.84±0.09
		%	145.4*	61.6*	236.9*	161.5*	49.7*	326.4*
5	30 days after TBI	M±m	6.12±0.10	3.02±0.06	2.02±0.08	5.97±0.16	2.69±0.11	2.22±0.10
		%	141.7*	59.1*	240.5*	150.0*	59.1*	255.2*

Note: * - the significant differences between groups as relative to control
CE - cholesterol; PL - phospholipids; TBI - traumatic brain injury

One day after the TBI reproduction the content of total PL in erythrocyte membranes decreased almost in 2 times (2.68±0.07 and 5.11±0.11 mmol/l in the control, P < 0,05), and the content of total CE in 1.5 times increased (6.59±0.17 at 4.32±0.15 mmol/l in the control P < 0,05), which led to an increase in the ratio of CE/PL in almost three times. Thus, it was found that TBI leads to marked changes in the content of lipids in erythrocyte membranes, and these changes were so severe that practically 30-days studies in the future did not reveal significant changes in their content. Even in the 30th day of study the content of total PL remained almost 2 times less than in the control, and the content of the total CE remained 1.5 times higher than in the control (table 2). Accordingly, the ratio of CE/PL remained in 2.5 times higher than in the control.

Even more pronounced were the changes in the dynamics of lipid content in the membranes of mitochondria of the cerebral cortex of rats, indicating the severity of brain damage (table 2). One day after the TBI reproduction, the content of total PL decreased by almost in 2 times (2.77±0.05 and 4.55±0.11 mg/g in the control, P < 0.05), and the total CE increased in 2 times (7.44±0.22 and 3.98±0.09 mg/g in control, P < 0.05). Accordingly, the ratio of CE/PL increased by more than in 3 times and reached 320.7 % of the control, P < 0.05. It should be noted that these changes remained until the 30th day of the study, with the appearance of a not significant tendency to normalization, but it was not quite statistically significant.

It is logical to investigate further changes in the content of lipids in combined pathology during reproducing TBI in

rats on the background of alcohol intoxication, which is a complicated and severe pathology [19]. The results indicate that already on the first day of the pathological state changes of the content of lipids in membranes of erythrocytes in rats were more pronounced than in the reproduction of separately alcohol intoxication or TBI (table 3).

Table 3. *Dynamics of total cholesterol content, total phospholipids and their molar ratio in rats under traumatic brain injury on the background of chronic alcohol intoxication and arbitrary recovery (n=7)*

№ n/n	Experiment conditions	Statistical indicators	Erythrocyte membranes (mmol/l)			Mitochondria membranes of the cerebral cortex (mg/g)		
			Total CE	Total PL	Ratio CE/PL	Total CE	Total PL	Ratio CE/PL
1	Control	M±m %	4.64±0.16 100.0	5.45±0.17 100.0	0.85±0.05 100.0	4.15±0.15 100.0	4.40±0.10 100.0	0.94±0.03 100.0
2	Alcohol intoxication (20 days) + TBI (1 day)	M±m %	7.99±0.22 172.2*	1.90±0.10 34.9*	4.20±0.12 494.1*	8.35±0.19 201.2*	1.59±0.05 36.1*	5.25±0.21 558.5*
3	Alcohol intoxication + TBI (10 days)	M±m %	9.01±0.24 194.2*	2.27±0.09 41.6*	3.97±0.13 467.0*	7.79±0.10 187.7*	1.79±0.06 40.7*	4.35±0.19 462.8*
4	Alcohol intoxication + TBI (20 days)	M±m %	8.72±0.19 187.9*	2.21±0.08 40.5*	3.94±0.11 463.5*	7.49±0.12 180.5*	2.11±0.09 47.9*	3.55±0.15 377.6*
5	Alcohol intoxication + TBI (30 days)	M±m %	7.68±0.18 165.5*	3.26±0.10 59.8*	2.35±0.10 276.5*	7.10±0.13 171.1*	2.20±0.10 50.0*	3.23±0.13 343.6*

Note: * - the significant differences between groups as relative to control
CE - cholesterol; PL - phospholipids; TBI - traumatic brain injury

Thus, the content of total PL decreased by almost by three times (to 1.90±0.10 and 5.45±0.7 mmol/l in the control, $P < 0.05$), and the total CE increased by more than in 1.5 times (7.99±0.22 and 4.64±0.16 mmol/l in control, $P < 0.05$). Their ratio was changed catastrophically from 0.85±0.05 to 4.20±0.12, by 5 times ($P < 0.05$). On the 30th day of observation the content of total PL increased almost by 2 times, but did not reach the control values (59.8%, $P < 0.05$), and the content of total CE almost did not change compared to the first day observation (7.99±0.22 at 7.68±0.18 mmol/l in the control, $P < 0.05$). As a result, the ratio remained high and discoordination remained stable and high. Changes that are even more striking were observed in membranes of mitochondria of the cerebral cortex of rats. On the first day of the study total PL decreased by almost three times, and total CE increased twice. Discoordination or ratio of CE/PL reached 558.8% compared with control, $P < 0.05$ (table 3). By the end of the observation period on 30th day the content of these lipids were somewhat aligned, but were far from reference values. The obtained results indicate that the combined pathology, which is TBI on the background of alcohol intoxication, leads to serious consequences, which are the violation of the content of lipids in membranes.

The following results were obtained and there was a logical question, how did combined pathology change the spectrum of isolated phospholipids? Further study of the phospholipid spectrum of erythrocyte membranes in rats under traumatic brain injury on the background of alcohol intoxication showed that this combined pathology not only significantly changed the content of total PL, but also substantially redistributes the content of their individual fractions. To date, the boundaries of the content of individual PL fractions have been thoroughly studied. However, their role is very diverse not only with various functional symptoms of the body, but also with various pathological manifestations. Unfortunately, these data are quite controversial, ambiguous and to make qualitative generalizations is difficult for them [21]. On this basis, we focus on the main properties of PL. It is known, that PL contains alcohol, glycerol or sphingosine, free fatty acids and phosphoric acid [15]. Some of them contain nitrogen-containing compounds such as choline, ethanolamine, inositol, serine – PC, PE, PI, PS [15]. All PL are divided into 2 groups. I group form glycerophospholipids (PC, PE, PI, PS), the main function of which is the formation of lipid bilayer membranes, regulation of the activity of membrane enzymes through the maintenance of the stability, viscosity and permeability of membranes. Their important function is also to provide a calcium-dependent mechanism for the transfer of hormonal signal into the cells. This group also includes LPC, which as a result of hydrolysis by phospholipase A_2 forms prostaglandins and leukotrienes [15]. The latter one form the immune-biological properties of the organism. II group – sphingophospholipids, which include SM, which causes two main functions in the body – regulation of the transmission of the nerve impulse and internal homeostasis. In addition, the PL can be divided into light oxidized, which include PC and PE and hardly oxidized (lysoforms), to which in the first place refer LPC, SM [22].

Investigation of the dynamics of the content of phospholipid fractions in this work we conducted on membranes of erythrocytes of rats with combined pathology, taking into account the greater availability of erythrocytes

and laborious method of determining individual fractions of PL (table 4). Studies have shown that for the quantitative content in the control of individual PL membranes of erythrocytes are located as follows: 35.9% occupied by PC; 20.4% - PE; 18.0% - SM; 8.6% - PI; 7.2% - LPC; 4.5 % - PS and 5.4 % - PAP. Thus, we can conclude that the number in the PL most commonly are PC, PE and SM. Their share accounts for 74.3%: it can be assumed that in the functional plan their participation in regulation of viscosity, permeability of membranes (PC, PE) and receptors ability (SM) support is more important. One day after the TBI reproduction on the background of alcohol intoxication, almost twice times pronounced decreasing in the content of total PL, a sharp discoordination of various PL fractions was observed. The number of PC and PE were decreased by almost by 3.5 times, and PI and PS were decreased almost by 2 times. At the same time, the number of LPC were increased almost by 2 times. Increasing the content of SM was statistically significant, but was only 117.5 % (table 4). These data are objective evidence of the above assumption. It is also necessary to emphasize the fact that more than a threefold decrease was attributed to PC and PE, which belong to the class of light oxidized PL, and PE and PS, in addition, contain polyunsaturated fatty acids. The content of hardly oxidized PL, such as LPC and SM, on the contrary increased. These data reflect the fact that the increase in LPC content indicates the activation of phospholipase A_2 and, consequently, an increase in the number of prostaglandins and prostacyclin formation [15]. This fact confirmed the discoordination of immune-biological protection of the body, increasing of catabolic and destructive processes in the erythrocyte membranes.

In subsequent monitoring periods, there was a gradual restoration of the content of total PL and their individual fractions, but at 30th day, it did not reach control values. Despite the significant increase in the level of total PL, on the 30th day of the study after the reproduction of TBI on the background of alcohol intoxication, the level of PC, PE and PI remains significantly lower compared to control, and the content of LPC and SM statistically significant were high. The content of the PS was close to the control values. That is, the discoordination of the metabolism of individual fractions of PL was so pronounced that even on the 30th day of arbitrary recovery did not reach the ascending values, which again emphasizes the seriousness of the morphofunctional state disturbance of membranes of red blood cells.

Conclusions

1. Studies have shown that chronic alcohol intoxication in rats and reproduction of an experimental traumatic brain injury separately causes a significant change in the content of total phospholipids and total cholesterol, and their molar ratio in the membranes of erythrocytes and mitochondria of the cerebral cortex. Particularly pronounced were these changes in the studied objects in combined pathology, that is, at the traumatic brain injury on the background of chronic alcohol intoxication.

2. Discoordination of lipid metabolism was unidirectional and consisted of a significant reduction in the content of total phospholipids and increased total cholesterol, which led to a violation of the molar ratio of CE/PL in the direction of increasing the coefficient, which is normally is about 1.0.

3. The results of the work show that the arbitrary restoration of the studied parameters during chronic alcohol intoxication on 30th day of observation significantly approached the control values, and when reproduced TBI, where the violations were more pronounced, on 30th day did not return to the ascending values. Under combined traumatic brain injury on the background of chronic alcoholic intoxication on 30th day the shift in lipid content only gained a marked tendency to normalize. That is, the discoordination of the lipid content was quite pronounced.

4. Along with the changes in the content of total PL, total CE and their molar ratio under combined TBI on the background of chronic alcohol intoxication in the membranes of erythrocytes, there was a significant shift of the spectrum of individual fractions of phospholipids. After 1 day of reproduction, the content of PC and PE decreased in 3.5 times, and in 2 times the PI and PS, at the same time, the content of LPC increased by almost 2 times.

5. A threefold decrease in the content of PC and PE, light oxidized PL, indicates a violation of the formation of lipid bilayer of membranes of erythrocytes and the activity of embedded in them enzyme systems due to changes in viscosity and penetration of membranes.

6. In subsequent monitoring periods there was a gradual restoration of individual fractions of PL, but on the 30th day of the study, it did not reach the control values. That is, the discoordination of the metabolism of individual fractions of PL was so pronounced that even on 30th day of arbitrary reproduction did not reach the ascending values, which again emphasizes the seriousness of the morphofunctional state disturbance of membranes of erythrocyte membranes.

Prospects for further research.

An important task for the future research should be the further study of the role of phospholipids in the formation of the effects of traumatic brain injury on the background of chronic alcohol intoxication and development of the methods of pharmacological correction of targeted action.

Declaration of conflict of interests

Authors certify the absence of conflict of interests.

Authors' contributions

All authors contributed equally to the research, data analysis, and writing of the manuscript. Final manuscript was read and approved by all authors.

Table 4. *Dynamics of content of phospholipid fractions in erythrocyte membranes of rats under traumatic brain injury on the background of chronic alcoholic intoxication and arbitrary recovery (mg / 100 ml of erythrocytes, n = 7).*

№ n/n	Experiment conditions	Statistical indicators	Total PL	Fractions of PL (%)						
				Phosphatidylcholine (PC)	Phosphatidylethanolamine (PE)	Phosphatidylinositol (PI)	Lysophosphatidylcholine (LPC)		Sphingomyelin (SM)	Phosphatidic acid phosphatase (PAP)
1	Control	M±m % (%TFPL)	275.5±4.1 100.0 100.0	98.9±3.1 100.0 35.9	56.2±2.4 100.0 20.4	23.7±1.0 100.0 8.6	19.8±1.4 100.0 7.2	12.3±0.9 100.0 4.5	49.6±1.4 100.0 18.0	15.0±0.9 100.0 5.4
2	Alcohol intoxication (20 days) + TBI (1 day)	M±m % (2-1) (%TFPL)	164.8±1.8 59.8* 100.0	28.8±1.8 29.1* 17.5	16.1±1.1 28.6* 9.8	12.7±0.8 53.6* 7.7	38.6±1.5 194.9* 23.4	7.4±0.8 60.2* 4.5	58.3±1.5 117.5* 35.4	2.9±0.2 19.3* 1.7
3	Alcohol intoxication + TBI (10 days)	M±m % (3-1) (%TFPL)	175.9±1.9 63.8* 100.0	29.0±1.7 29.3* 16.5	15.9±0.9 28.3* 9.0	14.1±0.9 59.5* 8.0	39.7±1.3 200.5* 22.6	8.5±0.7 69.1* 4.8	61.7±1.7 124.5* 35.1	7.0±0.4 46.7* 4.0
4	Alcohol intoxication + TBI (20 days)	M±m % (4-1) (%TFPL)	193.8±2.1 70.3* 100.0	35.2±1.8 35.6* 18.2	16.7±0.8 29.7* 8.6	13.9±0.7 58.6* 7.2	45.8±1.5 231.3* 23.6	9.7±0.9 78.9* 5.0	66.3±1.6 133.7* 34.2	6.2±0.3 41.3 3.2
5	Alcohol intoxication + TBI (30 days)	M±m % (5-1) (%TFPL)	214.5±2.8 77.8* 100.0	51.9±2.2 52.5* 24.2	40.3±1.4 71.7* 18.8	12.8±0.6 54.0* 6.0	31.2±1.2 157.6* 14.5	10.5±0.6 85.4* 4.9	59.7±1.5 120.4* 27.8	8.1±0.4 54.0* 3.8

Note: * - The difference between two groups (such as an experiment vs. control group) is judged to be statistically significant when p = 0.05 or less.
% - total fraction of phospholipids (TFPL) - fraction percentage relative to total PL

References

1. Конопля А.И., Локтионов А.Л., Дудка В.В. Хроническая интоксикация этанолом: метаболические изменения, коррекция нарушений. Токсикологический вестник, 2015; 5: 25-30.
2. Тесленко О.А. Клинико-диагностические и терапевтические особенности алкогольной энцефалопатии. Український неврологічний журн, 2013; 2: 105-117.
3. Tayebati S.K. Phospholipid and Lipid Derivatives as Potential Neuroprotective Compounds. *Molecules*, 2018 Sep; 23(9): 2257.
4. Engel K.M., Griesinger H., Schulz M., Schiller J. Normal-phase versus reversed-phase thin-layer chromatography (TLC) to monitor oxidized phosphatidylcholines by TLC/mass spectrometry. *Rapid Commun Mass Spectrom*, 2019; 33 (1): 60-65. doi: 10.1002/rcm.8237
5. Дерюгина А.В., Иващенко М.Н., Игнатьев П.С., Самоделкин А.Г. Трансляционные исследования электрофоретической подвижности и фазового портрета эритроцитов с учётом развития стрессовой реакции в условиях патологического процесса. Альманах клин медицины, 2018; 46 (8): 765-771.
6. Гречко А.В., Молчанов И.В., Сергунова В.А., Козлова Е.К., Черныш А.М. Дефекты мембран эритроцитов у пациентов с нарушениями функции головного мозга (пилотное исследование). Общая реаниматология, 2019; 15(6): 11-20. doi: 10.15360/1813-9779-2019-6-11-20.
7. Шерстюкова Е.А., Иноземцев В.А., Козлов А.П., Гудкова О.Е., Сергунова В.А. Атомно-силовая микроскопия в оценке механических свойств мембран эритроцитов при воздействии различных физико-химических агентов. Альманах клин медицины, 2021; 49 (6): 427-434.
8. Пахомова Р.А., Гуликян Г.Н. Атомно-силовая микроскопия эритроцитов больных панкреонекрозом. Сибирское медицинское обозрение, 2022; 2: 49-55. doi: 10.20333/25000136-2022-2-49-55.
9. Полозова А.В. Морфоструктурные особенности и функциональное состояние эритроцитов при моделировании черепно-мозговой травмы и её экспериментальной коррекции цитопротекторами. Дис. канд. биол. наук: 14.00.25. – Н. Новгород, Россия, 2021; 115.
10. Draeger A., Monastyrskaya K., Babiychuk E.B. Plasma membrane and cellular damage control: the annex in survival kit. *Biochem. Pharmacol*, 2011; 81(6): 703-712.
11. Кнышова Л.П., Поройский С.В., Яковлев А.Т., Морковин Е.И., Тарасов А.С. Критерии достоверности воспроизведения экспериментальной модели хронической алкогольной интоксикации. Волгоградский научно-*медицинский* журн, 2016; 4 (52): 48-51.
12. Цымбалюк В.И., Кочин О.В. Экспериментальное моделирование черепно-мозговой травмы. Украинский нейрохирургический журн, 2008; 2: 10-12.
13. Камышников В.С. Методы клинических лабораторных исследований. МЕДпресс-информ, Москва, Россия, 2016; 736.
14. Веселова Т.А., Веселов А.П., Дерюгина А.В. Тонкослойная хроматография липидов. ННГУ, Н. Новгород, Россия, 2015; 23.
15. Уилсон К., Уолкер Дж. Принципы и методы биохимии и молекулярной биологии. Лаборатория знаний, Москва, Россия, 2021; 848.

16. Петрова П.А. Сравнительный анализ методов исследования активности Mg^{2+}-зависимой Na^+/K^+-активируемой АТФ-азы в тенях эритроцитов. *Siberian Journal of Life Sciences and Agriculture*, 2017; 9(4-2): 150-166. doi:10.12731/wsd-2017-4-2-150-166.
17. Наконечная О.А., Стеценко С.О., Маракушин Д.І., Вишницька І.А. Вплив простих поліефірів на фосфоліпідний склад і активність ферментних мембранозв'язаних комплексів головного мозку щурів. Експериментальна *і* клінічна медицина, 2013; 1 (58): 33-36.
18. Костишин Н.М., Гжегоцький М.Р., Рівіс Й.Ф. Жирнокислотний склад загальних ліпідів і фосфоліпідів м'язової тканини та головного мозку щурів під впливом вібраційних коливань. *Zaporozhye medical journal*, 2016; 3 (96): 108-116.
19. Кресюн В.Й., Соколик О.П. Вплив черепно-мозкової травми на тлі хронічної алкогольної інтоксикації в експерименті на активність Na^+/K^+-АТФази еритроцитів та можливість її корекції. Одеський медичний журн, 2019; 1 (171): 27-33.
20. Li H., Xu W., Jiang L., Gu H. Lipidomic signature of serum from the rats exposed to alcohol for one year. *Toxicol Lett*, 2018 Sep; 15 (294): 166-176.
21. Esposito E., Cordaro M., Cuzzocrea S. Roles of fatty acid ethanolamides (FAE) in traumatic and ischemic brain injury. *Pharmacol Res*, 2014 Aug; 86: 26-31. doi: 10.1016/j.phrs.2014.05.009.
22. Traynor-Kaplan A., Kruse M., Dickson E.J. et al. Fatty-acyl chain profiles of cellular phosphoinositides. *Biochim Biophys Acta Mol Cell Biol Lipids*, 2017; 1862(5): 513-522. doi: 10.1016/j.bbalip.2017.02.002.

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