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ORIGINAL ARTICLE

Phospholipid composition of blood plasma and internal organs of rats with diclofenac-induced hepatitis

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Non-steroidal anti-inflammatory drugs, in particular sodium diclofenac, are characterized by direct cytotoxic action on hepatocytes in long-term use and in high doses. Therefore, the preparations of this group are used in experimental medicine and veterinary medicine for simulation of toxic liver damage in laboratory animals in determining the therapeutic effectiveness of preparations of hepatoprotective profile. Numerous cellular functions depend on the physicochemical properties of their lipid bilayer, the basis of which are phospholipids (PL). In the work the features of changes in the PL-composition of blood plasma and internal organs (liver, kidneys, heart and lungs) in Wistar line rats for artificial reproduction of toxic hepatitis at oral administration in the body of sodium diclofenac in a dose of 12.5 mg/kg body weight, one once a day, for 14 days. Thus, in patients with rats, there is a decrease in blood plasma content of both total (TPL) and individual PL, namely: phosphatidyl ethanolamine (PE) and sphingomyelin (SM). In addition, these animals reduce the content of TPL in most internal organs: in the liver - by 21%, in the heart - by 19% and in the lungs - by 28%. Against this background, the content of phosphatidylserine (PS) and phosphatidylinositol (PI) decreases in the liver, in the heart - PS, PI, phosphatidic acid (PA) and cardiolipin (CL), in lungs - phosphatidylcholine (PC), PE and SM, and in the kidneys - PE and SM (increased content of lysoforms), proving the destructive effect of the drug on the cell membrane. Consequently, for diclofenac-induced hepatitis in rats, the similarity of quantitative changes in both the TPL and individual representatives in blood plasma and internal organs is established, which is expressed in the formation of their deficiency level and is important for the elucidation of the molecular foundations of the development of the pathological process. In general, this indicates the expediency of finding biologically active substances that stimulate the adaptation processes in cells for the negative effects of xenobiotics and compensate for the imbalance of PL in the development of hepatopathology.

Keywords: phospholipids; blood plasma; liver; kidneys; heart; lungs; laboratory rat; experimental toxic hepatitis; sodium diclofenac

Introduction

To date, a sharp increase in the incidence of toxic liver damage has been recorded in medicine and veterinary medicine, of which up to 40% is due to medications (Donnelly et al., 2017; Lin et al., 2017). The majority of hepatitis and cirrhosis of the liver not established by etiology is also caused by the use of medications (Verbeek et al., 2015). Among drugs with direct cytotoxic effects on hepatocytes, long-term use and at high doses are nonsteroidal anti-inflammatory drugs (NSAIDs), in particular sodium diclofenac (Calderon et al., 2010; Basavraj et al., 2012; Abd Elazem and Seham, 2015). Therefore, the use of this group of drugs for the modeling of drug-induced liver damage in animals is used in pharmacological experiments in determining the therapeutic efficacy of hepatoprotective profile preparations (Melnychuk and Gryshchenko, 2016).

Most NSAIDs after absorption in the gastrointestinal tract enter the liver, which specifically responds to taking medications. In hepatocytes, they undergo biotransformation primarily under the action of cytochrome P450, 2E1 and 1A2 isoenzymes followed by the formation of N-acetylbenzoquinonimine (NAPQI) (Fabbrini and Magkos, 2015; Cole et al., 2016). The following steps in the conversion of NSAIDs are associated with the interaction of their derivatives with glutathione. In this case, hydrophilic metabolites formed are transported by membrane-bound proteins of hepatocytes into bile or blood. From the body they are removed through the digestive tract and kidneys. With increasing daily dose of the drug, the content of NAPQI is increased. In this case there is a deficit of glutathione. NAPQI interacts with nucleophilic groups of hepatocyte proteins, causing necrosis. Induction or inhibition of hepatic enzyme activity under the action of xenobiotics leads to an increase or decrease in the concentration of the latter in the plasma and, as a consequence, to the development of undesirable reactions, primarily inflammation.

In the pathogenesis of the toxic effect of NSAIDs, the intensity of lipid peroxidation (LPO) is also increased, which occurs against the background of the growing formation of free radicals, covalent binding of electrophilic metabolites to proteins, oxidation or a decrease in the content of free glutathione. These disorders in turn can lead to the following functional and morphological changes: an increase in the content of cytosolic Ca^{2+}, which activates phospholipase and proteinases and is

able to inhibit the attachment of microfilaments to the plasma membrane of the hepatocyte; polymerization of actin, which also leads to the destruction of microfilaments. As a result of the accumulation of LPO products, the lipid bilayer of membranes is damaged in the cell, accompanied by a decrease in the content of PL in them with simultaneous increase in the level of cholesterol. Now the key role of lipid bilayer membrane disorders in the development of severe liver pathologies, cardiovascular and nervous systems, disorders of many blood cell functions, and the like has already been proven.

The interaction of the pathological factor with the surface of the plasma membrane of cells triggers a cascade of interrelated biochemical processes that take place both on the membrane and inside the cells. The intensity of restoration of intracellular homeostasis in the course of development of pathology essentially depends on the duration of action of the pathogenic factor and the adaptive capabilities of the organism to a large extent determined by the degree of damage to the cell membranes. It is known that numerous cellular functions, such as enzymatic activity, hormonal response and permeability of membranes, depend on the physicochemical properties of their lipid bilayer, based on PL. So, the functioning of cellular membrane systems depends on the structural organization of their phospholipid molecules. It should be noted that PL biosynthesis is a complex multi-stage process, influenced by factors of exo- and endogenous media. The individual stages of the biosynthesis of various representatives are closely intertwined, which complicates their fine regulation (Gula and Margitich, 2009).

Thus, studies of the PL composition of tissues and animal organs will help determine the characteristics of pathogenetic changes in the structural organization of the main functional cells of the body important in determining the therapeutic tactics of hepatopathology therapy, as well as in developing and testing the reparative efficacy of newly created drugs. Therefore, the purpose of our work was to determine the characteristics of the PL composition of blood plasma and internal organs of rats in experimental hepatitis against the background of diclofenac sodium.

Materials and methods of research

In the experiments, rats (males) of the Wistar line with an average body weight of 200-220 g were attracted. The animals were divided into two groups (control and experimental) according to the principle of analogues of 12 animals in each. Two weeks before the start of the experiment, they were kept in quarantine with a daily clinical examination and monitoring of body weight and feed intake. The rats had free access to water and feed.

In working with animals, the requirements of the "European Convention for the Protection of Vertebrates used for experimental and scientific purposes" (Strasbourg, 1986) and the Law of Ukraine "On the Protection of Animals against Cruel Treatment" No. 3447 of 21.02.2006.

The rats of the experimental group artificially reproduced the drug form of toxic hepatitis (diagnosed for the results of clinical, biochemical, pathoanatomical and histological studies) by oral administration of a 5% solution of diclofenac (produced in tablets of OJSC "Khimfarmzavod Krasnaya Zvezda", Kharkov) at a dose of 12,5 mg/kg body weight (more than 10 times the therapeutic weight), once a day, for 14 days (Serdyukov et al., 2008; Melnychuk and Gryshchenko, 2016). In the control group, intact animals were administered to which an equivalent volume of distilled water was orally administered. The experience period was 14 days.

Clinical examination of patients with rats was carried out according to the following indices: behavior, appetite, body weight, skin condition and coat, including examination and palpation of the abdominal wall, characterized stool masses. Clinical symptoms of the disease began to appear in the animals of the test group as early as the 6th-7th day of oral administration of diclofenac sodium and were noted by general oppression, impaired appetite, a stable decrease in the average body weight in the group by 14-16 g, a dull fur coat, a decrease in skin elasticity, increased tactile sensitivity of the abdominal wall, liquid, unpleasant smell of feces with remnants of undigested food and impurities of mucus.

Experimental reproduction of toxic liver damage is characterized by a decrease in the metabolic and functional activity of hepatocytes, manifested by hypoproteinemia, hypoalbuminemia, hypoglycemia, hypolipidemia, hypocholesterolemia and is confirmed by high values of thymol sample, described in our previous work (Gryshchenko, 2017). At the same time, these animals were diagnosed with disorders of pigmentary liver function, development of hepatocellular insufficiency, cytolytic syndrome and biliary obstruction, which is confirmed by hyperenzymeemia of aminotransferases, alkaline phosphatase and γ-glutamyltranspeptidase.

When patho-anatomical dissection of rats with diclofenac liver damage, dark cherry color, flabby consistency and blood filling of the body is noted, signs of its dystrophy are established, and in animals of the control group it was distinguished by a uniform reddish-brown color, elastic consistency. Histological examination of liver slices in patients with rats shows an expansion of the blood vessels and their blood overflow, separate cells in the state of fatty degeneration (the nuclei are shifted to the periphery of the cell, the cytoplasm is transparent, the cells have a cricoid form), pronounced edema of the disses, discollection of hepatic beams and lymphocytic infiltration connective tissue (Serdyukov et al., 2008). The latter testifies to the development of the inflammatory reaction when diclofenac is administered to the rats in the experimental group.

Thus, the established macroscopic and histological changes in the liver in the animals of the experimental group characterize the development of nonspecific reactive hepatitis. From histo-morphological changes in the liver parenchyma, fat and granular dystrophy, loss of radial orientation of the platelets of hepatocytes and expansion of the disses, vasoconstriction are noted.

The blood in the rats was taken from the abdominal aorta into test tubes with heparin, then centrifuged at 1500 rpm for 15 min and the plasma was separated. Further studies were carried out on individual PL of plasma of blood, liver, lungs, heart and kidneys. Extraction of lipids from blood plasma and homogenates of liver, lung, heart and kidney samples was carried out

by the method (Folch et al., 1957). Chromatographic separation of individual PLs was carried out by two-dimensional thin-layer chromatography on standard plates of the firm "Sorbfil" (Vaskovsky et al., 1975) in a solvent system: chloroform-methanol-benzene ammonia (65:35:10:6) and chloroform-methanol-benzene Acetic acid-water-acetone (70:30:10:5:1:4). The content of individual PL was investigated by the amount of inorganic phosphorus P(neorg) using a molybdate reagent method (Vascovsky et al., 1975). Individual PLs were identified by specific reactions (Vlizlo et al., 2012) and their markers, and the content spectrophotometrically at Specol-II at λ 830 nm using a calibration curve constructed from P (neorg).

The experimental data were processed by conventional methods of variational statistics. The mean arithmetic values (M) and the error of the average statistical value (m) were calculated, which is represented in the tables in the form (M ± m). To determine the significant differences between the mean values, the Student's test (t) was used. Changes in the indices were considered reliable at $p < 0.05$.

Results and discussion

At the heart of irreversible cell damage is a violation of the functions of the plasma and intracellular membranes, the main lipid component of which is PL. That is why when modeling in the laboratory rats of a medicamentous form of toxic hepatitis against the background of diclofenac sodium application, a study was made of the qualitative and quantitative changes in individual PL of blood plasma and internal organs of rats, the results of which are presented in Tables 1-3.

Table 1. Phospholipid composition of blood plasma of rats with diclofenac-induced hepatitis, mmol/l (M ± m, n=12).

Index	Control	An experience
Total phospholipids	0.84 ± 0.07	0.62 ± 0.06*
Phosphatidylcholine	0.34 ± 0.05	0.25 ± 0.04
Phosphatidylethanolamine	0.23 ± 0.02	0.18 ± 0.01*
Phosphatidylserine	0.05 ± 0.01	0.04 ± 0.01
Sphingomyelin	0.07 ± 0.01	0.03 ± 0.01*
Phosphatidylinositol	0.09 ± 0.02	0.07 ± 0.02
Phosphatidic acid	0.03 ± 0.01	0.02 ± 0.01
Lysophosphatidylcholine	0.02 ± 0.001	0.02 ± 0.003
Lysophosphatidylethanolamine	0.01 ± 0.001	0.01 ± 0.001

Note: Here and in Table 2. 3 * - $p<0.05$, the results are probable in comparison with the values in the control group of rats.

According to the results given in Table 1, in the blood plasma of the rats of the experimental group. the content of both 26% of the TPL, as well as the representatives of individual PL, in particular. the PE and SM, by 22 and 57%, as well as the tendency to decrease the PC content by 26%, decreases both in comparison with the control. Regularities have been established with respect to quantitative changes in the PL spectrum of rat blood plasma, patients with drug-induced hepatitis, indicate the development of a deficient level of PL in their bodies. As is known (Gula and Margitich, 2009), this can lead to increased stiffness and microviscosity of cell membranes, microcirculation disturbance and a decrease in their permeability, indisputably disrupts the functioning of membrane structures. disrupts the vital activity of cells of the whole organism and can contribute to the development of pathological processes.

Therefore, the next stage of our study was to determine the specific features of changes in the phospholipid organization of the internal organs of rats with toxic liver damage.

As can be seen from Table 2, in the liver of the rats of the experimental group, as compared with the control. there is also a decrease in the content of TPL (by 21%), which occurs against the background of a decrease in the level of individual individual PL, in particular PS and PI, by 28 and 41%, respectively. This may be a consequence of a violation of their endogenous synthesis in hepatocytes. In spite of the fact that these PL belong to minor fractions, but they are biologically active signaling compounds that act as regulators of processes that are interrelated with intracellular metabolism.

Based on the data of Table 2 in the kidneys of the rats of the experimental group, compared with the control. the level of individual individual PL decreases, in particular, PE - by 19%. SM - by 13% and increase in the content of LPE by 26%. This may be due to the hepatotoxic effect of NSAID diclofenac not only on the functional cells of the liver, but also on the kidneys. At the same time. the growth in kidney tissue of LPE content is probably a consequence of destructive changes in PE.

Table 2. Phospholipid composition of liver and kidney in rats with diclofenac-induced hepatitis, mg/100 g of crude tissue (M ± m, n=12).

Index	Liver		Kidneys	
	Control	An experience	Control	An experience
Total phospholipids	94.27 ± 4.72	74.71 ± 3.60*	79.88 ± 5.97	69.25 ± 3.35
Phosphatidylcholine	43.79 ± 3.47	36.30 ± 2.93	35.30 ± 3.10	30.44 ± 2.15
Phosphatidylethanolamine	20.50 ± 2.76	15.21 ± 2.15	21.05 ± 1.58	17.00 ± 0.61*
Phosphatidylserine	16.08 ± 1.19	11.61 ± 1.28*	12.85 ± 1.20	11.70 ± 0.89
Sphingomyelin	5.60 ± 0.50	4.58 ± 0.21	4.60 ± 0.17	4.02 ± 0.10*
Phosphatidylinositol	1.75 ± 0.11	1.03 ± 0.06*	1.10 ± 0.03	1.05 ± 0.06
Phosphatidic acid	1.65 ± 0.25	1.53 ± 0.19	1.19 ± 0.09	1.10 ± 0.11

Lysophosphatidylcholine	2.61 ± 0.18	2.56 ± 0.23	2.39 ± 0.20	2.32 ± 0.11
Lysophosphatidylethanolamine	1.51 ± 0.12	1.22 ± 0.17	1.10 ± 0.08	1.38 ± 0.04*
Lysophosphatidylinositol	0.78 ± 0.10	0.67 ± 0.15	0.30 ± 0.05	0.24 ± 0.04

According to the data of Table 3 in the lungs of the rats of the experimental group, a 28% decrease in the content of TPL and individual individual PLs, in particular, of PC, PE and SM, by 30%, 40 and 38%, respectively, is observed compared with the control, which may indicate a violation of their synthesis in the tissues of the body. Quantitative changes in the lipid component of membranes of hepatocytes, in particular, most represented in their structure PL - PC and PE, leads to a violation of their structural and dynamic properties, including, microviscosity and permeability, which certainly negatively affects the trophism of cells and even more on their functional state.

Table 3. Phospholipid composition of lungs and heart in rats with diclofenac-induced hepatitis, mg/100 g of crude tissue (M ± m, n=12).

Index	Lungs		A heart	
	Control	An experience	Control	An experience
Total phospholipids	20.21 ± 0.33	14.51 ± 0.26*	83.70 ± 4.26	68.11 ± 3.77*
Phosphatidylcholine	7.89 ± 0.23	5.55 ± 0.09*	28.05 ± 3.02	22.15 ± 0.99
Phosphatidylethanolamine	6.55 ± 0.58	3.95 ± 0.21*	19.78 ± 2.80	16.08 ± 1.88
Phosphatidylserine	0.46 ± 0.06	0.35 ± 0.08	4.26 ± 0.19	3.17 ± 0.27*
Sphingomyelin	0.55 ± 0.08	0.34 ± 0.05*	4.43 ± 0.16	4.28 ± 0.25
Phosphatidylinositol	2.00 ± 0.07	1.92 ± 0.10	3.92 ± 0.25	2.40 ± 0.14*
Phosphatidic acid	1.09 ± 0.11	0.86 ± 0.13	3.57 ± 0.31	2.26 ± 0.25*
Cardiolipin	-	-	13.55 ± 0.46	12.02 ± 0.31*
Lysophosphatidylcholine	-	-	1.69 ± 0.10	2.08 ± 0.08*
Lysophosphatidylethanolamine	0.80 ± 0.20	0.79 ± 0.17	1.85 ± 0.09	1.78 ± 0.07
Lysophosphatidylinositol	0.87 ± 0.14	0.75 ± 0.10	2.60 ± 0.29	1.89 ± 0.23

At the same time, in the heart of the rats of the experimental group, as compared to the control (see Table 3), the content of both TPL by 19% and individual individual PLs, in particular PS, PI and PA, decreased by 26%, 39 and, respectively 37%, as well as an increase in the content of LFH by 23%. At the same time, the level of an important component of cardiomyocyte membranes, cardiolipin (CL), is reduced by 11%, which is a structural component of the inner mitochondrial membrane and is necessary for the functioning of numerous membrane-bound enzymes involved in energy metabolism. It is known that the precursor of CL and other glycerophospholipids in mammalian cells is PA, and the latter is synthesized on the outer mitochondrial membrane. From there, the PA passes to the internal mitochondrial membrane, where it acts as a precursor of the CL synthesis (Gula and Margitich, 2009). Since in patients with animals there is a simultaneous decrease in the content of the heart muscle in the cardiac muscle and, possibly, is correlated with this fact of PA level decrease, it is obvious that mitochondrial dysfunction in cardiomyocytes is developed in diclofenac-induced hepatosis. Although the endoplasmic reticulum occupies a leading place in the synthesis of CL, mitochondrial dysfunctions can adversely affect the regulation of lipid homeostasis. In turn, the increase in the level of lyso-PL (in particular, LPH) to cytotoxic concentrations may be a consequence of disturbance of the process of deacetylation/re-alkylation of PL. Thus, the accumulation of LPI in acute myocardial ischemia is an important factor in the pathogenesis of cardiac arrhythmias (Gula and Margitich, 2009).

Physiological regeneration of the heart muscle occurs at the intracellular level with a high intensity, as for cardiomyocytes, their wear and tear is characteristic. Accordingly, by reducing the level of PL in the heart, its regenerative capacity is disturbed, which is directly related to changes in their ratio in the membranes of cardiomyocytes.

So, as a result of the evaluation of the results of the PL spectrum of blood plasma and internal organs (liver, kidneys, lungs, heart) of laboratory rats, it should be noted that when simulating experimental hepatitis in these animals against the background of diclofenac sodium, significant changes in the content of both TPL, and individual individual PLs, which, on the whole, are characterized by their decrease (Figure 1). These data on the changes in the quantitative ratio of individual PLs characterize the development of cellular insufficiency at the membrane level, which in turn leads to a disruption of the structural organization of vital internal organs, primarily the liver, which certainly has a negative effect on the functional state of the whole organism.

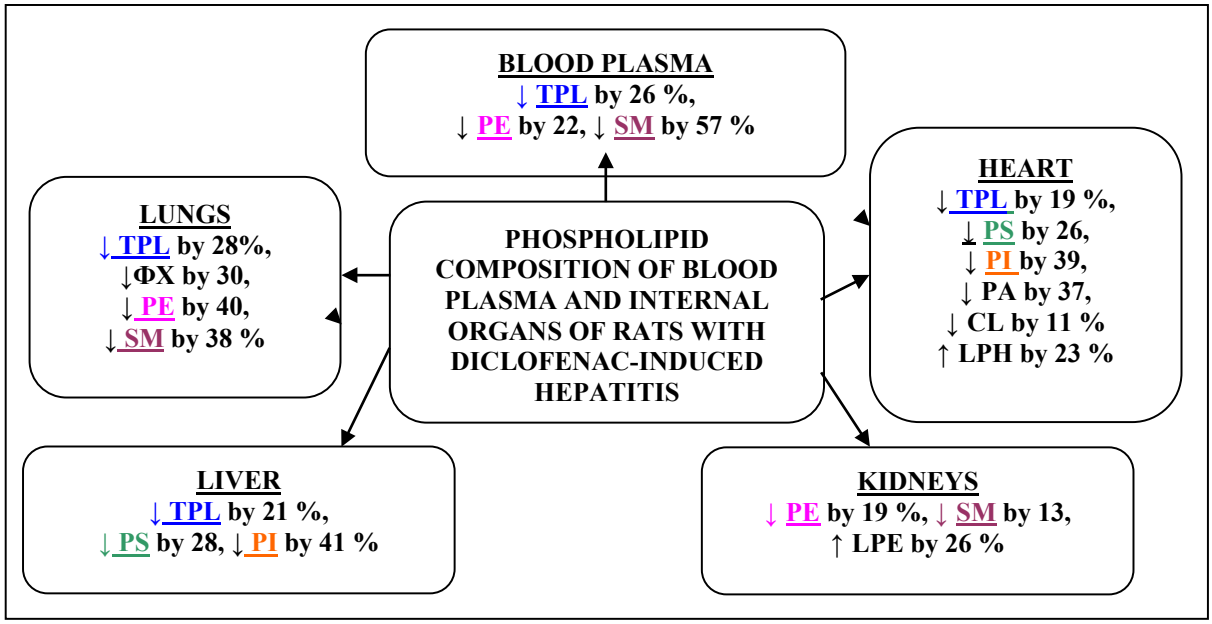

Figure 1. Regularities of changes in the phospholipid composition of blood plasma and internal organs of rats with diclofenac-induced hepatitis.

A decrease in the internal organs tissues (liver, heart) in the tissues of the PS can be used as an indicator of apoptosis, Such cells are absorbed by phagocytes. At the same time there is a deficiency in the tissues of PI (liver, heart), which plays an important role in intracellular signaling processes. Among other PLs, the content of PC (lungs) and PE (plasma of blood, lungs, kidneys), which are the main structural components of cell membranes, is substantially reduced, and therefore this can negatively affect their functions and cell integrity. These PL are metabolically related to each other and provide stabilization of the plasmolemia structure and membranes of cellular organelles. Reduction of the content of these PL in biological membranes can be a factor that leads to a disruption of their ultrastructural organization, a change in biosynthetic processes, loss of individual metabolic links, a decrease in the level of vital energy-dependent processes, active ion transport, etc. All this leads to an increase in the permeability of cell membranes and , respectively, violations of their transport functions, is a well-known universal sign of cell damage,

Changes in the PI content lead to changes in the rate and direction of metabolic processes, since this PL participates in active transport of substances through cellular membranes (Melnychuk and Gryshchenko, 2005). At the same time, changes in the content of SM are possible due to activation or suppression of the synthesis of SM with PC.

Unbalanced changes in the distribution of PL in the damaged tissues of various organs and, above all, the liver cause violations of specific functions involved in the pathological process of cells. Attention is drawn to the fact that despite the tendency of the content of the main structural PLs of cell membranes (PC and PE) to decrease in the studied organs, significant changes occur with minor fractions (PS, PI), as well as SM, which are biologically active, signaling substances capable of compensating for the metabolic imbalance arising in response to the toxic effects of xenobiotic. In this case, the accumulation of detergent lyso-PL in the tissue of the kidney and heart against the background of a decrease in the content of both major and minor fractions of PL is a fundamental biochemical mechanism of transformation of one species into another that underlies the adaptation of the organism at the cellular level. Therefore, lipid imbalance is important in terms of the molecular basis for the development of the pathological process.

So, as a result of experimental modeling of toxic hepatitis in rats, features of changes in the PL composition of blood plasma and internal organs have been established, proving the destructive effect of sodium diclofenac on cell membranes,

Conclusions

1. In patients with toxic hepatitis of rats against the background of diclofenac sodium administration, a significant decrease in the plasma content of both 26% TPL and individual individual PL was found, namely, PE and SM, respectively, by 22 and 57%, compared with the control that indicates the development in their body of a deficient level of these structural lipids. The latter may lead to an increase in stiffness and microviscosity of cell membranes, a violation of microcirculation and a decrease in their permeability, unquestionably disrupts the functioning of membrane structures, disrupting the vital activity of the cells of the whole organism.

2. Modeling of toxic hepatitis in laboratory rats also leads to a decrease in the content of TPL in most internal organs, namely: in the liver - by 21%, in the heart - by 19 and in the lungs by 28%, which agrees with the established regularity in plasma blood and indicates the formation of a deficient level of structural lipids in the organs under investigation. At the same time, in this liver, the content of PS and PI is reduced by 28 and 41%, respectively, in the heart of PS, PI, PA and CL, by 26%, 39, 37 and 11%, respectively (LPC level is increased by 23%), in the lungs, PC, PE and SM, respectively, by 30%, 40 and 38%, and despite the absence of significant changes in the TPL in the kidneys, a deficient level of individual individual PLs is formed, namely, PE and

SM, respectively, by 19 and 13% (the content of LPE increases 26%), which proves the destructive effect of diclofenac sodium on cell membranes.

3. In diclofenac-induced hepatitis in rats, the similarity of the quantitative changes of individual individual PLs was established, In particular, PE and SM decrease quantitatively in blood plasma, kidneys and lungs, and the content of minor fractions of PI and PS in the liver and heart, which may indicate some similarities in the mechanisms of structural and functional rearrangement in the cell membranes of the corresponding internal organs, which correlates with the manifestation of adaptive processes at the subcellular level in response to toxic liver damage by xenobiotics.

4. These data theoretically justify the expediency of searching for biologically active substances (BAS), which stimulate the course of cell adaptation compensate for the imbalance of PL in hepatopathology, After all, the PL storage of cell membranes determines its functional activity, and changes in their content and species spectrum in the animals cause disruption of the functioning of biomembranes, which can be both a consequence and a cause of pathology.

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