STRUCTURAL AND FUNCTIONAL STATE OF CYTOPLASMATIC MEMBRANES AS A MONITORING INDEX OF TOXIC INFLUENCE OF THE SIMPLE POLYETHERS ON ORGANISM

Kharkiv National Medical University, Kharkiv, Ukraine

Zaytseva O., Dr.Sci. (Biology), Professor

Zhukov V., Dr.Sci. (Biology, Medicine), Professor, Chief of Department of Biochemistry

Telegin V., PhD (Medicine), Associated Professor

Gramatiuk S., Associated Professor

Risovanaya L., Assistant

Radzishevskaya Yar., Doctor of internal medicine

Boiko A., Doctor of internal medicine

Abstract. *In subacute toxicologic experiment on the white Wistar rats it was investigated the effect of the simple polyethers of P-373-2-20; P-5003-AC and P-294-2-35 on the state of membrane phospholipid fractions, ionic permeability (velocity of free and induced exite of K^+) and the fluidity (by coefficient of the pirenic excimerization) of lymphocyte, erythrocyte, hepatocyte plasmatic membranes as well as free-radical processes, peroxidation of lipids and proteins. It was elicited the simple polyethers of P-373-2-20; P-5003-AC and P-294-2-35 in doses of 1/10, 1/100, 1/1000 DL_{50} can breach physical-chemical characteristics and structural-functional properties of cytoplasmatic membranes, stimulate free radical processes, speed up the protein-and lipoperoxidation. Dose of 1/10000 DL_{50} is uneffect.*

Key words*: toxycology, xenobiotics, cytoplasmatic membranes*

The plasmatic membrane is a primary object of the effects of chemicals in all the ways of their entry into the body, and the change of its structure will always be fraught with dysfunction of the cells and metabolism. According to many authors [1-3, 5], xenobiotics have the effect the

membrane, cause free radical pathology in the organism, suppress the cellular and humoral immunity, have mutagenic and embriotropic influences, reinforce the processes of atherogenesis, and others, which are based on a violation of the structural and functional state of biological membranes. This fully applies to the little-studied group of xenobiotics - polyethers grades P-373-2-20, P-5003-AC, P-294-2-35, which have found application in various sectors of the economy to produce plastics, foams, polyurethanes, epoxy resins, lacquers, enamels, hydraulic, coolant and brake fluids, emulsifiers, flotation reagents, etc.

The aim of the work was to study the structural and functional state of biological membranes in conditions of subacute effects on the organism by polyether grades P-373-2-20, P-5003-AC, P-294-2-35.

Materials and research methods. The experiment was conducted on 66 adult white Wistar rats with initial body weight 0,18-0,21 kg. The control and experimental groups were kept in vivarium conditions similar to the standard diet. The animals of experimental group daily (once) for 1.5 months orally using a metal probe is inserted aqueous solutions of polyethers of grades P-373-2-20 (DL_{50}=32.3g/kg), P-5003-AC (DL_{50}=36.2g/kg), P-294-2-35 (DL_{50}=14.8g/kg) at doses of 1/10 , 1/100, 1/1000 DL_{50}. In the study of super weak luminescence was used dose of 1/10000 DL_{50} also. Upon completion of the experiments the animals were killed by decapitation under light ether anesthesia. The research program included the determination of the status of membrane phospholipid fractions, ion permeability, viscosity, charge, polarity and fluidity of erythrocyte, hepatocyte and lymphocyte membranes, analysis of the free radical (FR) processes, lipid peroxidation and oxidative modification of proteins.

Influence of xenobiotics on protein and lipid components of membranes was assessed by the percentage of the fraction of phospholipids in membranes of erythrocytes, leukocytes, and lymphocytes by two-dimensional thin-layer chromatography. To study the phospholipid

composition it was determined levels of phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylserine (PS), lisophosphatidylethanolamin (LPEA), lysophosphatidylcholine (LPC), phosphatidylethanolamin (PEA), phosphatidylinositol (PI), phosphatidic acid (PhA), and cardiolipin (CL). Phospholipids were determined by inorganic phosphorus with the identification of standard solutions of lipids and phospholipids quality detector. To investigate the ionic permeability of erythrocyte membranes we used an indicator such as the speed of the free and induced release of K^+ ions. Change in viscosity, polarity and charge of the membranes under the influence of polyethers has been studied using fluorescent probes [3]. In assessing the fluidity of erythrocytes and lymphocytes membranes, pyrene excimerization coefficient was calculated, which varies in proportion to fluidity. It represents the ratio of pyrene excimer number at emission wavelength of λ_{em}=470 nm to the number of its monomers at the wavelength λ_{em}=393 nm. Pyrene excimerization coefficient was studied in the region of protein-lipid contacts at the excitation wavelength λ_{ex}=287 nm and in the lipid bilayer - λ_{ex}=334 nm.

To evaluate the free radical oxidation and lipid peroxidation (LPO) it was used a spontaneous (SChL) and induced (IChL) chemiluminescences and phosphorescence of blood serum, whole blood and homogenates of internal organs and tissues of experimental animals. The record of SChL and IChL was carried out using an automatic medical chemiluminometer ChL1F-01 [4]. The course of study: 1.2 ml of salt solution; 10 ml of serum (or 10 mkl of internal organs homogenates), 10 ml of 3% solution of luminol were injected in a quartz cuvette (size 10x10x45 mm) and background of superweak luminescence is recorded twice for 10 seconds. Then in a quartz cuvette for the assessment of IChL 3% hydrogen peroxide in an amount of 0.05 ml is injected and 6 measurements performed, each for 10 seconds. The study of the phosphorescence of blood serum was carried out on the luminometer (phosphoroscope). Peroxidation of proteins is studied by the method of [4], investigating the serum content of 2,4 -

dinitrophenilaldohydrosone (2,4-DNPh-A) (unit of optical density per 1 g protein, walelength λ=370 nm) and 2,4 - dinitrophenilketohydrosone (2,4-DNPh-K) (unit of optical density per 1 g protein, walelength λ = 380 nm).

Statistical data processing was performed using the software package "STATISTISA 6.0 WINDOWS". The reliability of differences was evaluated by the Student – Fischer indices at the level of $p<0.05$. Experimental studies on animals were carried out in accordance with "International guidelines for biomedical research using animals" (Strasbourg, 1995), as well as national "General ethical principles of experiments with animals" (Ukraine, 2001).

Results of research and their discussion. The research results of FR processes and LPO are given in Table 1. Apparently xenobiotics in doses of 1/10, 1/100 and 1/1000 DL_{50} increased in subacute experience the intensity of H_2O_2-induced and luminol-induced BChl of homogenates of internal organs and tissues. The dose of 1/10000 DL_{50} didn't effect on the level of BChl intensity. It should be noted that there were increased contents of malondialdehyde (MDA) and diene conjugates in serum and liver in the experimental groups of animals at doses of 1/10 and 1/100 DL_{50} ($p <0.05$).

Table 1. Effect of polyether-dose 1/100 DL_{50} on the BChL intensity and the content of LPO products

Indicators, research facilities	Substances M ± m			
	control	P-373-2-20	P-5003 AC	P-234-2-35
Diene conjugates (nmol / ml), serum	2,48 ±0,17	3,80 ±0,26*	4,20 ±0,35*	4,10 ±0,30*
MDA (nmol / ml), serum	0,75 ± 0,08	1,52 ±0,15*	1,48 ±0,17*	1,60 ± 0,16
Diene conjugates (nmol / g), liver	4,80 ± 0,25	7,60 ± 0,28*	8,20 ± 0,22*	8,40 ± 0,36*
MDA (nmol / g), liver	2,20 ± 0,16	3,85 ± 0,14*	4,10 ±0,25*	4,50 ± 0,32*
Luminol-induced. BChl (imp / s), serum	770,31 ± 20,62	1240,32 ±38,61*	1350,41 ±30,51*	1286,21 ±22,30*
Luminol-induced. BChl (imp / s), liver	860,40 ± 27,21	1296,21 ±37,40*	1395,01± 43,52*	1325,41 ± 33,60*
H_2O_2-induced. BChl (imp / s), serum	720,32 ± 26,8	980,41 ± 27,90*	1053,51 ±23,82*	1010,60 ± 19,82*
H_2O_2-induced. BChl (imp / s), liver	810,50 ± 19,70	1204,08 ±22,60*	1248,31 ±31,50*	1205,21 ±28,60*

Note: * - differences statistically significant from control, $p< 0,05$

Analysis of the data shows that the subtoxic effects of the studied polyethers at all doses except 1/10000 DL_{50} induce FR processes and LPO, which is known to be accompanied by generation of reactive oxygen species and accumulation in the organism of hydroperoxides, peroxides and free radicals that can lead to inhibit-ion of the activity of antioxidantic system (AOS) and the development of pathological structural and metabolic states. Increased levels of intensity of luminol-induced and H_2O_2-induced BChL confirmed series character of FR changes in biological systems, which are then activate the lipid peroxidation. Studies show that in the intoxication of xenobiotics superoxide radical anion of oxygen ($O_2^{\bullet}$) and hydroxyl radical ($OH^{\bullet}$) are formed. Moreover, the first of which indicates the presence of high levels of excited electronic states (triplet), it is obviously due to the change in the conformation of the protein molecules present in serum.

The study of the phosphorescence intensity of blood serum of experimental animals revealed significant differences in their values at the excitation wavelengths λ = 297; 313; 334; 365; 404 and 434 nm. Particularly significant the phosphorescence increase was in long-wavelength (λ = 434 nm) and shortwavelength (λ = 297 nm) regions of excitation. The appearance in the long-wavelength region of excitation increased number of molecules in the triplet state is due, apparently, uncoupling of oxidative phosphorylation, and tissue respiration, which is accompanied by an increase of heat dissipation in the organism of experimental animals under the influence of xenobiotics. At a dose of 1/10000 DL_{50} changes of the phosphorescence of blood serum in the experimental group of animals was not observed with respect to control group.

As we see, under the influence of xenobiotics there is observed activation of oxidative processes that forms the development in the organism the dystrophic and destructive violations in the cellular and intracellular structural and functional units.

Subacute effect of polyethers leads up to increased concentrations in blood serum aldo- and ketohydrosones - products of oxidative modification of proteins (Fig. 1). The accumulation of these products was significant in groups of animals subjected to doses of 1/10 and 1/100 DL_{50}. At a dose of 1/1000 DL_{60} significant differences from control were not observed.

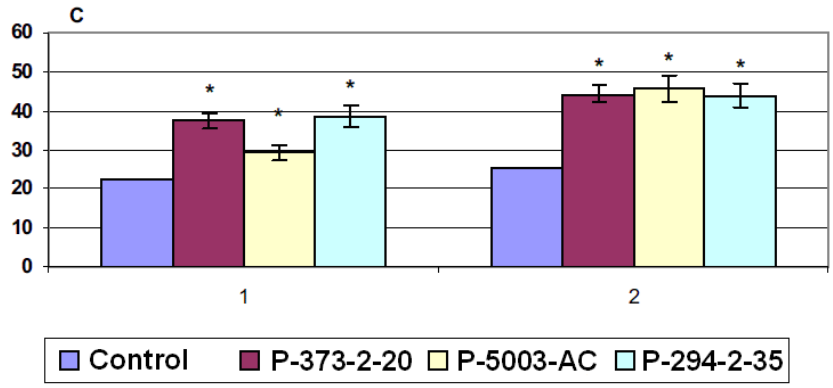

Fig. 1. Effect of polyethers at a dose of 1/100 DL_{50} on content of proteins peroxidation products concentration of (C, units. of optical density / g protein) phospholipids in membranes of erythrocytes and leukocytes. 1) $\lambda = 370$ nm, 2) $\lambda = 380$ nm.

Note: * - differences statistically significant from control, $p< 0,05$.

Since the polyethers contain hydrophilic and hydrophobic groups of radicals, we can assume probability of a priority of their influence on protein and lipid components of membranes. In this context, determination of the concentrations of phospholipid fractions in membranes of erythrocytes and leukocytes by two-dimensional thin-layer chromatography was carried out.

These data clearly demonstrate that the investigated xenobiotics not only activat lipid peroxidation, but peroxidation of protein structures also.

As the results of studies, xenobiotics in doses of 1/10 and 1/100 DL_{50} alter the percentage of almost all the studied fractions of the membrane phospholipids (Table 2).

Table 2 Effect of polyether P-294-2-35 at dose of 1/100DL_{5O} on the percentage of phospholipid fractions

Indices (%)	Cell membranes, M ± m			
	Erythrocytes		Leukocytes	
	Control	Test	Control	Test
PEA	20,4 ±1,82	14,65 ± 0,72*	24,5 ± 1,64	15,24 ±0,67*
PC	41,32 ±1,56	58,42 ±1,65*	38,9 ±1,37	60,75 ± 1,86*
SM	14,73 ±1,33	9,86 ± 0,57*	17,3 ±0,65	10,42 ± 0,66*
PS	11,56 ± 0,71	7,24 ± 0,43*	9,1 ±0,58	8,73 ± 0,46
LPEA	1,22 ± 0,32	3,15 ±0,26*	1,4 ±0,25	2,76 ± 0,20*
LPC	1,35 ± 0,25	4,28 ± 0,35*	1,2 ± 0,15	3,40 ± 0,27*
PI	6,27 ± 0,53	3,56 ±0,18*	7,4 ±0,65	4,25 ± 0,38*
CL	0,52 ± 0,04	0,88 ± 0,05*	0,54 ± 0,06	0,77 ± 0,06*

Note: * - differences statistically significant from control, $p< 0,05$.

It was revealed that the effect of xenobiotics on membrane phospholipids of various tissues was similar. In all cases, the substances reduced the content of PEA, SM, PI, and increased levels of PC, LPC, LPEA and CL. Common and characteristic feature of these changes in the structure of membranes is the appearance of lizoforms of phospholipids - LPC and LPEA, which served as important evidence of structural violations and the emergence of highly toxic metabolites of lipid metabolism. However, it should be noted that in the membrane fractions of leukocytes were given less significant changes than in the erythrocyte membranes, which is due in all probability, with their low level of repair and synthetic processes occurring in the membranes of the nuclear-free cells.

In the course of the experiment it was revealed that in the experimental group of animals at the end of subacute experience xenobiotics led up to a decrease in fluidity (pyrene excimerization ratio) of the cytoplasmic membrane of blood cells (erythrocytes and leukocytes) compared with control. This process were more susceptible to erythrocyte membranes, in which

significant changes are set in the lipid bilayer and in the area of protein-lipid contacts. In a dose-dependent effects of xenobiotics, membrane fluidity was reduced to 50% (Fig. 2).

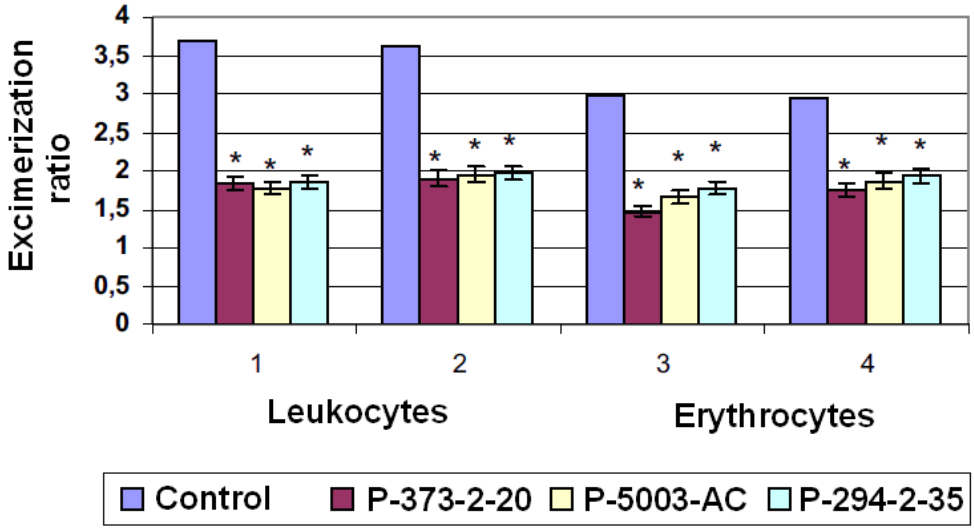

Fig. 2. Effect of polyether at a dose of 1/100 DL_{50} on fluidity (excimerization ratio) of erythrocyte and leukocyte membranes. 1;3 - protein-lipid contacts: 2;4 - lipid bilayer.

Note: * - differences statistically significant from control, $p< 0,05$.

Research of 1 - anilin-8-naphtalinesulphate (1,8-ANS, a fluorescent probe) fluorescence intensity in lymphocytes and erythrocytes, reflecting the change in surface charge of membranes revealed its a substantial reduction in experimental groups of animals. In a dose-dependent decrease in fluorescence intensity of exposure was in the range of 30 to 95%. Analysis of the literature shows that the decrease in fluorescence may be associated with an increase in the polarity of the membranes due to dehydration of protein molecules and the accumulation of water in the membrane structures. Prolonged exposure of polyethers in a subacute experiment was accompanied by a profound violation of the physical and chemical properties of membranes, including the ion permeability.

Studies have shown that polyethers in doses of 1/10 and 1/100 and 1/1000 DL_{50} increased spontaneous and induced by valinomycin K^+ ion yield from the red blood cells, also in combination with the previously detected changes indicating a violation of the structural and

functional organization of their membranes (Table 3). Streams of spontaneous release of K^+ ions increased in comparison with the control group by 5-10 times depending on the dose of exposure. It was found that more intensive xenobiotics influenced on spontaneous recovery of K^+ from erythrocytes. To a lesser extent the rate varied valinomycin-induced release of K^+ ions, which figures in the experimental group exceeded the control of only 2-2.3 times.

Table 3. The influence of polyethers at 1/100 DL_{50} on spontaneous and induced output of K^+ from erythrocytes.

Substances	Studied parameters, M ± m (million / ml)		
	The rate of spontaneous release of K^+ ions from erythrocytes	The rate of induced by valinomycin K^+ ion yield from the erythrocytes	The total number of K^+ ions per 1 mm of erythrocytes
Control	0,54 ± 0,03	6,45 ± 0,24	17,98 ±1,13
P-373-2-20	4,75 + 0,32*	12,68 ±0,87*	84,53 ± 3,75*
P-5003-AC	5,43 ± 0,36*	14,20 + 0,57*	90,84 ± 5,68*
P-294-2-35	6,10 + 0,45*	16,35 ±0,93*	93,26 ±6,10*

Note: * - differences statistically significant from control, $p< 0,05$

Couclusions. Thus, analysis of the study of the influence of polyethers on the structural and functional state of the membranes allowed the following conclusions:

1. In the subacute oral exposure polyethers P-373-2-20, P-5003-AC, P-294-2-35, in doses of 1/10, 1/100, 1/1000 DL_{50} stimulate free radical processes, lipid peroxidation and oxidative modification of proteins.
2. Polyethers in the indicated doses break the physical and chemical characteristics and structural and functional properties of the cytoplasmic membranes - their polarity, permeability, fluidity, hydrophobic volume, which inevitably involves a changes in the intracellular metabolism and the formation of dystrophic and destructive changes in various organs and tissues.
3. Xenobiotics in a dose of 1/10000 DL_{50} has no effect on the structural, functional, and physicochemical properties of the membranes of blood cells.

List of references

1. Жуков В.И., Попова Л.Д.. Зайцева О.В. Простые и макроциклические эфиры: научные основы охраны водных объектов – Харьков: Торнадо, 2000.-437с.

2. Жуков В.И., Резуненко Ю К, Зайцева О.В. Тормозные и гидравлические жидкости. Гигиенические аспекты охраны окружающей и производственной среды. – Харьков, 1999.-255с

3. Зайцева О.В. Поверхностно-активные вещества как стимуляторы свободнорадикальных процессов. J. Environment & Health, 2000; 2(13): 8-11.

4. Зайцева О.В.. Жукова Н З . Броше Е.А. Состояние свободнорадикальных процессов, перекисного окисления липидов и белков при псориазе. Ж. Експерим. і клінична медицина, 2002: 4:86-39.

5. Курляндский Б. А. Медицинские проблемы химической безопасности России в аспекте современных международных тенденций. Современные проблемы токсикологии, 2002; 3: 31-34