Effect of oligomers on structural and functional state of biomembranes

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

Knigavko V., Dr.Sci. (Biology), Professor, Chief of Department of Medical and biological Physics and Medical Informatics

Gramatiuk S., Associated Professor

Risovanaya L., Assistant

Abstract. *The effect of a new group of oligomers on the structural and functional state of erythrocytes, lymphocytes, hepatocytes, splenocytes membranes of rats under subacute experiment conditions was investigated. It is established that the oligomers are able to alter the membrane physical-chemical properties and disrupt the structural and metabolic processes that underlie the formation of pathological states.*

Key words: *membranes, phospholipids, lipid peroxidation, ion transport, fluorescent probes.*

Oligomers based on ethylene and propylene oxides widely are used in various sectors of the economy. Large volumes of their production create a potential hazard to human health [1,2]. Particular attention is forecasting the biological activity of new groups of compounds acquiring toxicodynamics, toxicokinetics and biotransformation research, in which the leading role belongs to the study of structural and functional state of cell membranes [3].

The purpose of this study was to investigate the influence of a new group of oligomers based on ethylene and propylene oxides on the structural and functional state of

erythrocytes, lymphocytes, splenocytes and hepatocytes membranes in the subacute experiment.

Materials and research methods. Work was realized on Wistar rats weighing 150-180 g, which daily for 1.5 months with the help of the probe oligomers: polyoxipropyleneglykol L-202, polyoxipropylenetetramethilenglykol L-1102-4-80, polyoxiethyleneoxipropylenethriols L-3003 -2-60 and L-4003-2-20 were injected at doses of 1/10, 1/100, 1/1000 DL_{50}. L-202 has DL_{50}=3040.0 mg/kg; L-1102-4-80 has DL_{50}=4800.0 mg/kg; L-3003-2-60 has DL_{50}=3210.0 mg/kg; L-4003-2-20 has DL_{50}=5870.0 mg/kg of animal weight. In each group, there were 10-15 animals. The intact rats not injected by oligomers served as a control group. The research program included the determination of phospholipid fractions, ion permeability, viscosity, charge and polarity of the of erythro-, hepato-, spleno- and lymphocytes membranes. Analysis of the membrane phospholipids fractions was performed by two-dimensional chromatography. Identification of phospholipids was performed according to standard solutions and quality detectors. To detect violations of the membrane permeability we used method for measuring the rate of K^+ ions going into medium without K^+ with help of glass ion-selective electrode twice: the rate of spontaneous going and the velocity of K^+ ion yield induced by the valinomycin. Changes in membrane viscosity, polarity and charge under the influence of oligomers was studied using fluorescent probes. The intensity of free radical processes and lipid peroxidation (LPO) as significant indices of structural-functional state of the membranes was studied by the intensity of luminol- and H_2O_2-dependent biochemiluminescence (BChl), the accumulation of malondialdehyde (MDA) and diene conjugates (DC). Status of oxidative

modification of proteins was assessed by levels the aldo- and ketohydrozones. Statistical analysis was performed by the Student-Fisher t-criterion.

Results of research and their discussion. It is known that many xenobiotics can stimulate free radical processes and lipid peroxidation, create the accumulation in organs and tissues the peroxides, hydroperoxides and other reactive radicals having membrane disturbing property. Changes in the state of homeostasis is accompanied by shifts in metabolic processes in the organism, for study of which we used BChl method as the most sensitive. Oligomers in doses of 1/100 and 1/1000 DL_{50} increased BChl intensity of internal organs homogenates, as well as the contents of MDA and DC in the liver and serum (Table 1), the contents of aldo- and ketohydrozones, which indicates stimulation of oxidative modification of proteins (Table . 2). The dose of 1/10000 DL_{50} was inoperative.

Table 1. *The state of free radical processes and lipid peroxidation under the effect of oligoethers in dose of 1/1000 DL_{50}, (M ± m)*

Substance	DC		MDA		Luminol-induced. BChL, intencity I, imp/s		H_20_2-induced. BChL, intencity I, imp/s	
	serum, nmol / ml	liver, nmol / g	serum, nmol / ml	liver, nmol / g	serum	liver	serum	liver
L-202	4,9±0,2*	7,5±0,4*	1,4±0,2*	5,8±0,6*	1105,4±30,5*	1315,5±48,6*	920,4±31,6*	1094,3±27,5*
L-1102-4-80	3,8±0,2*	9,2±0,7*	1,7±0,2*	5,4±0,4*	1235,0±40,7*	1275,0±51,3*	895,6±21,9*	1103,8±30,6*
L-3003-2-60	4,1±0,3*	8,0±0,4*	1,4±0,2*	5,2±0,4*	1280,6±37,2*	1304,5±42,3*	980,3±27,5*	1120,6±22,4*
L-4003-2-20	4,2±0,3*	8,4±0,4*	1,5±0,1*	4,6±0,3*	1154,3±28,2*	1260,8±32,3*	925,6±17,2*	1136,2±38,4*
Control	2,5±0,2	4,9±0,3	0,7±0,1	2,4±0,1	780,6±21,3	865,7±30,5	710,3±25,4	805,6±23,7

Note: Here and in Table. 2-5 * - p <0.05 compared with control.

Table 2. *Effect of oligomers in dose of 1/10 DL_{50} on the contents of proteins peroxidation products, (M ± m), optic. density units / g protein*

Substance	2,4-	2,4-

	dinitrophenylaldohydrosone, λ = 370 nm	dinitrophenylketohydrosone, λ = 380 nm
L-202	37,82±2,16*	46,52±2,37*
L-1102-4-80	24,56±2,17	44,83±2,43*
L-3003-2-60	42,33±1,60*	41,78±1,96*
L-4003-2-20	39,80±2,70*	43,65±2,28*
Control	21,74±1,83	24,56±2,17

Taking into account the oligomers contain hydrophilic and hydrophobic radicals, we can assume a priority of their influence on protein and lipid membrane structure. In this regard, determined the percentage contents of phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamyne (PEA), lizophosphatidylethanolamyne (LPEA), lysophosphatidylcholine (LPC), phosphatidic acid (PA), cardiolipin (CL), and sphingomyelin (SM) in erythrocytes, leukocytes , splenocytes and hepatocytes. Oligomers in doses of 1/10 and 1/100 DL_{50} changed the percentage of almost all the studied phospholipids fractions. Common and characteristic change is the emergence of lizoforms (Table 3), which is evidence of violations of the membrane structure, accompanied by the formation of highly toxic compounds.

Table 3. *Effect of oligomers in dose of 1/100 DL_{50} on phospholipidic composition of cell membranes (M ± m), persentage (%) from the total quantity*

Substance		PEA	PC	SM	PS	LPEA	LPC	PA	CL
L-202	Erythrocytes	14,5±0,9*	58,6±1,7*	10,3±0,8*	7,2±0,6*	3,1±0,4*	4,4±0,7*	3,9±0,8*	0,75±0,04*
	Leukocytes	15,3±0,9*	61,4±2,7*	12,6±0,7*	8,90±0,6	4,30±0,3*	4,5±0,2*	4,10±0,35*	0,85±0,09*
	Hepatocytes	16,4±1,3*	60,2±2,3*	12,1±1,2*	9,2±0,7	4,00±0,28*	4,78±0,60*	3,70±0,25*	0,80±0,07*
	Splenocytes	17,0±1,9*	60,3±2,6*	13,4±0,9*	8,20±0,65	3,8±0,6*	3,5±0,4*	5,10±0,35*	0,90±0,08
L-3003-2-60	Erythrocytes	14,2±1,5*	58,3±2,6*	10,2±1,3*	7,4±0,5*	3,6±0,8*	4,20±0,45*	3,9±0,7	0,80±0,05*
	Leukocytes	14,8±1,2*	62,3±2,7*	11,5±1,3*	8,9±0,7	4,50±0,37*	4,3±0,4*	4,10±0,25*	0,80±0,06*
	Hepatocytes	15,4±1,6*	61,7±2,4*	11,3±1,7*	8,8±0,7	4,10±0,35*	4,85±0,73*	3,65±0,30*	0,75±0,09*

	Splenocytes	17,9±1,7*	59,4±2,8*	13,3±0,7*	9,5±0,9	4,95±0,60*	3,30±0,25*	2,8±0,4*	0,70±0,08*
Control	Erythrocytes	20,4±1,8	41,3±1,5	14,7±1,3	11,5±0,7	1,2±0,4	1,3±0,3	6,2±0,7	0,50±0,06
	Leukocytes	24,5±1,8	38,9±1,5	17,3±0,6	9,1±1,3	1,4±0,3	1,15±0,20	7,3±0,8	0,55±0,07
	Hepatocytes	23,3±2,1	39,3±3,1	16,0±0,9	9,0±1,2	1,3±0,6	1,1±0,2	7,7±0,9	0,5±0,1
	Splenocytes	21,3±1,6	40,3±2,4	15,2±0,8	9,2±0,9	1,4±0,3	0,8±0,1	6,8±0,5	0,45±0,08

However, the oligomers didn't violate the percentage content of PS in leukocytes, splenocytes, hepatocytes, whereas its content in erythrocytes was significantly decreased.

The most significant changes in the distribution of phospholipidic fractions are determined in red blood cells that is explained, apparently, by a low level of repair and synthetic processes in these nuclear-free blood cells. By the end of subacute experience under the oligomers influence there was the decrease of fluidity of the erythrocytes cytoplasmic membranes compared with control (Table 4). This process was significant in the lipid bilayer and in the area of protein-lipid contacts. Depending on the dose and duration of exposure of oligomers membrane fluidity was reduced up to 40%. In lymphocytes reduction of fluidity mainly was in lipid bilayer and the maximum was under dose of 1/100 DL_{50}.

Table 4. *Effect of oligoethers in dose of 1/100 DL_{50} on membrane fluidity of erythrocytes and lymphocytes (coefficient of excimerization λ_{em} = 470 nm / λ_{em} =393 nm) (M ± m)*

Substance	Lymphocytes		Erythrocytes	
	protein-lipid contacts	lipid bilayer	protein-lipid contacts	lipid bilayer
L-202	1,75±0,03*	1,90±0,04*	1,50±0,03*	1,70±0,03*
L-3003-2-60	1,80±0,04*	1,95±0,06*	1,60±0,05*	1,75±0,04*
L-1102-4-80	1,65±0,06*	1,80±0,05*	1,40±0,04*	1,60±0,03*
Control	3,75±0,09	3,60±0,06	2,95±0,04	2,93±0,06

In addition, oligomers increased the immersion of proteins in lipid bilayer of erythrocytes and lymphocytes membranes, which can lead to a breach of the membrane-bound enzymes activity. More significant changes in membrane fluidity and the immersion of proteins in the lipid matrix in red blood cells, compared with those in lymphocytes, are connected, apparently, with a low potential of the repair of damaged membranes. The fluorescence intensity of 1,8-ANC probe in lymphocytes and erythrocytes, which reflects changes in the surface charge of membranes, significantly decreased in the experimental groups at 32-94%, depending on the dose oligoethers. This can be due to increased polarity of the membrane as a result of dehydration of protein molecules and the accumulation of water in the membrane structures. All oligoethers in doses of 1/100 and 1/1000 DL_{50} increased spontaneous and induced by valinomycin K^+ ion yield of the red blood cells, that is evidence of the violation of the structural and functional membrane organization and, consequently, the ion transport (Table 5).

Table 5. *Effect of oligoethers in dose of 1/100 DL_{50} on spontaneous and induced output of K^+ from red blood cells ($M \pm m$)*

Substance	Speed of K^+ release, mln/min		Total amount of K^+ ions in 1 ml of erythrocytes, mln/ml
	Spontaneous	Valinomycin-induced	
L-202	3,65±0,23*	11,80±1,14*	88,95±3,70*
L-3003-2-60	4,26±0,35*	14,75±1,20*	83,45±4,60*
L-1102-4-80	5,30±0,62*	15,40±1,60*	90,30±3,55*
L-4003-2-20	4,80±0,43*	13,95±1,40*	85,60±4,15*
Control	0,53±0,04	6,50±0,27	18,95±1,62

Changing the phospholipid fractions distribution, accompanied by an increase of their lizoforms, and violation of physical and chemical parameters of the state of plasma membranes were the structural and metabolic basis of changes in transport function of biological membranes, which implies a violation of the metal ions flow and nuclear-cytoplasmic interactions.

Conclusive. Thus, the investigated group of oligomers has a unidirectional effect on the structural and functional state of membranes, accompanied by a change in their physico-chemical properties - polarity, charge, permeability, viscosity, hydrophobic volume, which can lead to qualitative and quantitative changes in the activity of metabolic processes in the structural and functional units of cells and disintegration of the nuclear-cytoplasmic interactions that underlie the formation of pathological conditions. The dose of 1/10 000 DL_{50} does not effect on the physicochemical and metabolic properties of the membranes.

List of reference.

1. Голиков С.Н., Санацкий И.В., Общие механизмы токсического действия. Л.: Медицина, 1986. – 276 с.
2. Жуков В.И., Кротенко Р.И., Резуненко Ю.К. и др. Медико-биологические аспекты проблемы охраны водных объектов от загрязнения поверхостно-активными веществами. Харьков: Торнадо, 2000. – 390 с.
3. Поливода Б.И., Конев А.В., Попов Г.А. Биофизические аспекты радиационного поражения биомембран. М.: Энергоатомиздат, 1990. – 268 с.