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Phospholipid Composition of Erythrocytes and Glutathione Redox System in Rats during Adaptation to Cholesterol Load

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We studied phospholipid composition of erythrocytes and the state of the glutathione redox system in rats during adaptation to cholesterol load for 180 days. The adaptive response is formed during the period from day 30 to day 90 of cholesterol load and is associated with increased relative content of phosphatidylethanolamine and phosphatidylinositol, activation of glutathione redox system enzymes (glutathione reductase and glutathione peroxidase), and enhanced production of glutathione.

Key Words: *phospholipids; glutathione redox system; adaptation; cholesterol-rich ration*

Numerous clinical, experimental, and epidemiological studies proved that unhealthy diet is a risk factor of alimentary-dependent pathologies, e.g. atherosclerosis, CHD, diabetes mellitus, steatohepatitis, *etc*. [3,4]. Deficit or excess of some nutrients (protein deficit, fat and carbohydrate excess, *etc.*) initially induces a cascade of stress reactions aimed at activation of adaptation mechanisms.

Cytoplasmic membrane and its phospholipids (PL) play the leading role in processes of cell adaptation [9,10]. Thus, interconversion of PL and their redistribution between the inner and outer layers provide the basis for an important adaptation mechanism maintaining structural organization and functional properties of the plasma membrane [9]. Modification of membrane phospholipids can be initiated by activation of LPO processes and accumulation of lipoperoxides [1]. An important role in antioxidant defense (AOD) against LPO products is played by glutathione redox system, which is regulated by NF-κB and antioxidant response element (ARE) [5,11]. Rapid adaptive response of the glutathione system consisting in activation of glutathione reductase and glutathione peroxidase and synthesis of glutathione prevents PL oxidation and disturbances of the lipid backbone of the membrane. Study of PL and glutathione redox system under conditions of cholesterol load extends our knowledge about some mechanisms of cell adaptation and disadaptation.

Here we studied PL composition of erythrocytes and the state of glutathione redox system in rats during adaptation to cholesterol load.

MATERIALS AND METHODS

Experiments were carried out on 40 mature male Wistar rats weighing 173.0±5.6 g. The animals were divided into 4 groups (10 rats per group): controls (intact

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rats) receiving standard ration and experimental animals receiving experimental ration for 30 (group 1), 90 (group 2), or 180 days (group 3). The animals were maintained in a vivarium in accordance with sanitary rules for organization, equipping, and maintenance of experimental biological clinics. Experimental hypercaloric atherogenic ration included melted beef fat and cholesterol (CH) (19 and 2% of total weight of the ration, respectively). The animals were decapitated under ether narcosis on days 30, 90, and 180 of the experiment. The blood was sampled after overnight fast from the cervical veins after decapitation.

Lipid spectrum of blood serum was studied on a FP-901 analyzer (Labsystems). Total CH, triglycerides, and HDL CH were measured. The atherogenic index (AI) was calculated by the formula: (total CH–HDL CH)/HDL CH [3]. Erythrocytes were used as a universal model of cell membrane. Lipids were extracted from erythrocytes as described previously [7]. Polar lipids were fractionated by two-dimensional micro-thin-layer chromatography on glass plates (6×6 cm) with fixed silica gel and gypsum layer. The content of each component was presented in percents of the total PL content.

The state of glutathione redox system in blood erythrocytes was analyzed by the content of reduced glutathione and activities of glutathione reductase (NAD(P)H:oxidized glutathione oxidoreductase, EC 1.6.4.2) and glutathione peroxidase (glutathione:H_2O_2 oxidoreductase, EC 1.11.1.7). The amount of reduced glutathione was determined by the method of Ellman, the calculations were performed using a calibration curve. Activity of glutathione reductase was evaluated by the rate of NAD(P)H oxidation in the presence of oxidized glutathione [6]. Activity of glutathione peroxidase was determined by changes in absorption of reduced glutathione after incubation in the presence of H_2O_2. The content of LPO products was measured by MDA content in blood erythrocytes in the reaction with 2-TBA.

Statistical significance of the differences between the means was evaluated using Wilcoxon, White, and Kolmogorov–Smirnov tests and Student *t* test (for normal distribution).

RESULTS

Feeding hypercaloric ration led to the formation of alimentary dyslipidemia. In group 1 rats, elevation of total CH, triglycerides, and atherogenic index and decrease in HDL CH were observed (Table 1). In group 2 rats, a decrease in triglyceride concentration was found, while atherogenic index in this group by 1.7 times surpassed the corresponding parameter in the control. In group 3 animals, total CH and atherogenic index increased.

Fractionation of erythrocyte PL from experimental rats revealed the presence of 6 components: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin, phosphatidylinositol, and phosphatidic acid (trace amounts).

In group 1 rats, redistribution of PL towards accumulation of phosphatidylserine and phosphatidylethanolamine and a decrease in the relative content of phosphatidylinositol and phosphatidylcholine was observed (Table 1). The deficit of PL forming the outer layer of the membrane lipid backbone, phosphatidylinositol and phosphatidylcholine, attest to activation of specific phospholipases and intensification of LPO promoting destruction of the plasma membrane [1,10]. Changes in the level of phosphatidylethanolamine primarily located in the inner membrane lipid leaflet containing active centers of membrane-bound enzymes are a protective mechanism responding to these shifts [9]. The assumption that increased concentration of phosphatidylethanolamine can be a result of compensatory reaction relies on published reports demonstrating recovery of ion-transporting function of purified Ca^{2+}-ATPase after addition of phosphatidylethanolamine in the composition of artificial lipid vesicles. Incorporation of phosphatidylethanolamine into membranes produced similar effect on Na^+,K^+-ATPase activity [1].

In group 2 rats, the shifts in the content of phosphatidylserine, phosphatidylcholine, and phosphatidylethanolamine in erythrocyte membrane were similar to those observed in group 1 rats. Phosphatidylcholine deficit in the outer layer of erythrocyte membrane was compensated due to maintenance of sphingomyelin at the control level and accumulation of phosphatidylinositol. The latter requires sufficient activation of AOD enzymes preventing oxidation of polyunsaturated fatty acids or enhanced degradation of products of their peroxidation by blocking free-radical chain reaction. This state can be characterized as a compensatory reaction of the cell to long-term exposure to alimentary stress-factors. Moreover, due to high unsaturation of phosphatidylethanolamine, CH does not incorporate into the inner membrane leaflet. This allows preserving the hydrophilic microenvironment and hence functional activity of integral membrane proteins [9]. The observed redistribution of the main classes of PL attests to profound structural rearrangement of the lipid backbone of the membrane, on the one hand, and represents an adaptive reaction under conditions of exogenous hypercholesterolemia.

The decrease in the content of phosphatidylcholine forming the outer leaflet of the cell membrane persisted also after 180-day hypercaloric diet. The decrease in the content of phosphatidylcholine and phosphatidylinositol in group 3 rats was accompanied by a significant increase in the content of phosphati-

TABLE 1. Serum Lipids and PL of Blood Erythrocytes from Rats at Different Terms of High-Cholesterol Diet ($M\pm m$; n=10)

Parameter	Control	Group 1	Group 2	Group 3
Total CH, mmol/liter	1.57±0.04	3.34±0.04***	1.68±0.08^{+++}	2.04±0.17*
Triglycerides, mmol/liter	1.12±0.04	1.95±0.06***	0.51±0.05***$^{+++}$	1.17±0.08
HDL CH, mmol/liter	0.67±0.04	0.26±0.02***	0.50±0.08^{+++}	0.50±0.15^{+++}
Atherogenic index	1.43±0.15	11.87±1.55***	2.46±0.35*$^{+++}$	7.4±0.8***$^{+++}$
Phosphatidylserine, %	6.80±0.85	13.00±0.67***	11.1±0.5***$^{+}$	13.01±1.04***$^{+}$
Phosphatidylinositol, %	3.90±0.01	1.14±0.17***	4.69±0.19*$^{+++}$	1.66±0.29***
Sphingomyelin, %	14.42±0.97	15.77±2.09	14.50±0.39	17.80±0.52***$^{+++}$
Phosphatidylcholine, %	55.88±1.14	42.38±0.96***	47.70±0.66**	46.40±2.37**
Phosphatidylethanolamine, %	21.50±0.75	28.82±0.99***	22.01±0.34^{+}	20.40±1.05^{++}

Note. Here and in Table 2: *p<0.05, **p<0.01, and ***p<0.001 compared to the control; ^{+}p<0.05, ^{++}p<0.01, and ^{+++}p<0.001 compared to group 1.

TABLE 2. MDA Content and Parameters of Glutathione Redox-System in Blood Erythrocytes from Rats at Different Terms of High-Cholesterol Diet ($M\pm m$; n=10)

Parameter	Control	Group 1	Group 2	Group 3
MDA, nmol/g Hb	4.6±0.3	5.3±0.3*	8.1±0.2**$^{+++}$	9.1±0.2***$^{+++}$
MDA, μmol/g Hb	5.3±0.2	3.5±0.3***	4.1±0.1*$^{++}$	2.0±0.2***$^{++}$
Glutathione reductase, μmol NADPH/g Hb/min	75.1±1.5	68.0±1.5**	73.3±1.6^{+}	50.1±2.4***$^{++}$
Glutathione peroxidase, μmol glutathione/mg Hb/h	44.5±0.8	32.5±1.3**	40.1±2.2^{++}	21.4±1.2***$^{+++}$

dylserine and sphingomyelin compared to the control group. CH is actively incorporated into sphingomyelin clusters in the membrane due to high degree of unsaturation of this PL, which reduces permeability of the cell membrane and impairs the processes of active transport and transfer of substances across the membrane [1,10]. On the one hand, this attests to structural and functional incompetence of the plasma membrane, and on the other hand, transient increase in sphingomyelin and CH content is essential for the maintenance of the bilayer structure and integrity. Saturation of the plasma membrane with phosphatidylserine can be mediated by increased occupation of scavenger-receptors [7], which impairs elimination of damaged cells and modified lipoproteins from the circulation and leads to intoxication and inflammation.

The decrease in the relative content of phosphatidylinositol in cell membranes can be caused by LPO activation. The increase in MDA concentration (by 13.4%, p<0.01) in rat erythrocytes was observed on day 30 of the experiment (Table 2). Activities of glutathione reductase and glutathione peroxidase decreased by 9 (p<0.01) and 26% (p<0.001), respectively, the content of reduced glutathione decreased by 35% (p<0.001).

On day 90 of the experiment, the level of reduced glutathione increased by 18% (p<0.01) compared to group 1, activities of glutathione reductase and glutathione peroxidase returned to the control levels. It is known that cell sensitivity of thiol disulfide balance increases during oxidative stress. Under conditions of impaired glutathione status, reactive oxygen species act as second messengers and activate regulatory genes (NFκB and ARE) expressing enzymes of the glutathione system and glutathione synthesis [11]. This compensates insufficient level of glutathione system components and low AOD. This mechanism preserves membrane lipid bilayer and prevents PL oxidation, membrane destruction, and cell necrosis.

On day 180 of alimentary load, hyperproduction of LPO substances was observed: MDA content in erythrocytes increased 2-fold (p<0.001) compared to the control group. The state of glutathione AOD system is characterized by profound inhibition of glutathione peroxidase and glutathione reductase. Activities of glutathione reductase and glutathione peroxidase decreased by 1.5 and 2.0 times, the level of glutathione decreased by 2.6 times (p<0.001 for all parameters), which attests to aggravation of the imbalance between

pro- and antioxidants. Under these conditions, the observed degradation of phosphatidylinositol, a marker of oxidative stress in erythrocytes of group 3 rats, is a possible cause of insufficiency of adaptation capacities of the glutathione AOD redox system and formation of a cellular pathology, which attests to failure of compensatory processes.

All these findings suggest that the increase in phosphatidylserine (membrane-structuring PL) and phosphatidylinositol (most metabolically important PL fraction), suppression of LPO processes due to activation of enzymes of glutathione AOD redox-system, and enhanced glutathione synthesis are important mechanisms of adaptation of the cell membrane to cholesterol load. Under conditions of cholesterol load, the adaptive response in the cell is actively formed and realized during the period from day 30 to day 90. It can be hypothesized that exhaustion of compensatory mechanisms in the glutathione AOD system and LPO intensification lead to profound rearrangement of cell membrane lipid matrix on day 180 of lipid load. The obtained results extend our understanding of the mechanisms of cell adaptation and disadaptation under conditions of stress exposures and can provide the basis for creation of therapeutic and diagnostic technologies of cell pathology.

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