Structural and Functional Characteristics of Erythrocyte Membranes and Their Correction with Perftoran

N. B. Karmen, N. P. Milyutina, and A. A. Orlov

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We studied the effect of single intravenous injection of perftoran on the intensity of the initial stages of lipid peroxidation and structural and functional characteristics of erythrocyte membranes (cell model). Perftoran slightly activated the initial stages of lipid peroxidation and optimized structural and functional characteristics of erythrocyte membranes.

Key Words: *lipid peroxidation; antioxidant system; extraerythrocytic hemoglobin; microviscosity; polarity*

A key role in the pathogenesis of hypoxic and reoxygenation injury is played by imbalance between the pro- and antioxidant systems that modulates various cell functions including induction of apoptosis or necrosis. Perftoran (PF), an oxygen-transporting compound, can modulate these processes. The intensity of lipid peroxidation (LPO) in erythrocyte membranes serves as a criterion for this process. H. Heckers and D. Platt (1988) revealed common structural characteristics of membranes in erythrocytes and other cells in the organism. A correlation was found between variations in membrane characteristics of erythrocytes and others cells (*e.g.*, in internal organs).

MATERIALS AND METHODS

Experiments were performed on 28 male Wistar rats weighing 200-250 g. The animals were divided into 4 groups (7 rats per group). Group 1 rats served as the control. Group 2, 3, and 4 animals intravenously received PF 24, 72, and 168 h before blood sampling, respectively. PF was injected into the caudal vein. The blood was stored at 4°C for 24 h.

The contents of malonic dialdehyde (MDA) and conjugated dienes, activities of superoxide dismutase (SOD) and catalase, and concentration of extraerythrocytic hemoglobin (EEH) were measured using Reanal kits.

Microviscosity of membrane lipids (F_E/F_M (334)) was measured at an excitation wavelength of 334 nm. Microviscosity of protein-lipid contact sites (F_E/F_M (282)) was estimated at excitation maximum of 282 nm. The degree of protein immersion into the lipid bilayer was determined by protein fluorescence quenching with pyrene (F_0-F/F_0) due to radiationless energy transfer from tryptophanyl residues to pyrene. The measurements were performed at an excitation maximum of 282 nm. Polarity of pyrene environment in the membrane was estimated by the F_{372}/F_{393} ratio. It was calculated as a ratio between the intensity of pyrene fluorescence in the erythrocyte suspension at an excitation maximum of 334 nm and fluorescence wavelengths of 372 and 393 nm. Structural characteristics were studied in erythrocyte suspension diluted to 0.720 optical density units at an absorption wavelength of 650 nm. The final concentration of pyrene was 8 μM. Fluorescence spectra were recorded on a Hitachi-650-60 fluorescence spectrophotometer.

RESULTS

Single intravenous injection of PF moderately increased in the intensity of LPO in erythrocyte membranes (Table 1).

The concentrations of primary and end LPO products surpassed the normal at different terms of the

Laboratory for Thermodynamics and Energetics of Compound Biological Systems, Institute of Experimental and Theoretical Biophysics, Russian Academy of Sciences, Pushchino. ***Address for correspondence:*** karmen@_iteb@mail.ru. N. B. Karmen

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experiment. It should be emphasized that the ratio of LPO end-products in erythrocyte membranes (MDA and, especially, Schiff bases) was below the control. The observed changes had a positive role, because accumulation of highly toxic LPO end-products contributes to blockade of ion channels, membrane receptors, and other membrane proteins. This process is usually irreversible [3]. The initial stage of LPO accompanied by accumulation of conjugated dienes is a reversible process that produces a modifying effect.

Moderate activation of LPO in erythrocyte membranes was accompanied by an increase in catalase activity. Enzyme activity reached 167.4, 127.7, and 165.7% of the control value 24, 72, and 168 h after administration of PF, respectively ($p<0.01$). Three days after PF injection catalase activity slightly decreased (by 23.7% compared to day 1 postinjection), but remained above the control value (by 27.7%, $p<0.05$). Under these conditions SOD activity did not differ from the control (Fig. 1).

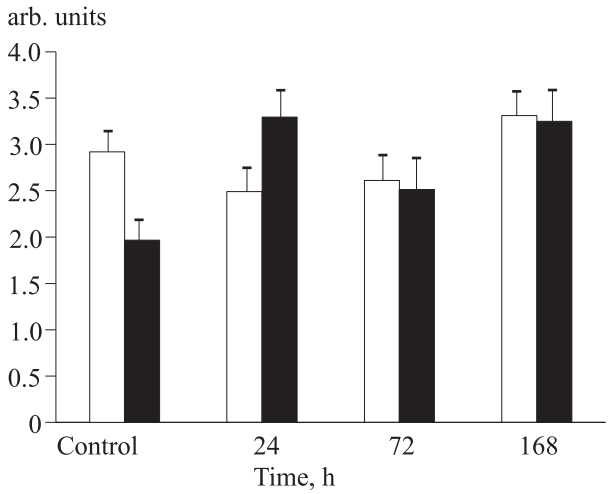

Fig. 1. Activities of SOD (light bars) and catalase (dark bars) in the control and after perftoran administration.

The study of structural and functional characteristics of erythrocyte membranes showed that PF increases their stability (Table 2). This conclusion was derived from changes in pyrene excimerization coefficient F_E/F_M (334), which characterized fluidity of the membrane lipid layer and remained unchanged in various periods after treatment. However, pyrene excimerization coefficient F_E/F_M (282) characterizing fluidity of the lipid layer in the zone of annular lipids (protein-lipid contact sites) was by 22.0-41.8% below the control ($p<0.05$). These changes reflect increased structuredness of the annular lipid zone, which can modulate function of membrane enzymes, receptors, and ion channels. It can be hypothesized that perfluorodecalin is dissolved in the membrane lipid phase, increases structuredness of annular lipids, nonspecifically interacts with membrane proteins, and elevates their functional activity [2].

PF slightly decreased the ratio of F_{372}/F_{393} (334), which reflects a decrease in aqueous corrosion and permeability of membranes [1]. Similar changes were observed in the ratio of F_{372}/F_{393} (282).

Plasma EEH concentration in treated rats that serves as the major criterion of erythrocyte membrane stability was lower than in the control (Fig. 2). Previ-

TABLE 1. LPO Intensity in Erythrocyte Membranes ($M\pm m$)

Parameter	Control	Time after PF injection, h		
		24	72	168
Conjugated dienes, nmol/mg lipids	2.23±0.53	2.30±0.23	3.56±0.41***	3.86±0.35*
MDA, nmol/mg lipids	3.53±0.25	4.01±0.27	4.57±0.28**	4.26±0.39
Schiff bases, rel. units/mg lipids	0.563±0.057	0.413±0.045***	0.605±0.081	0.684±0.040*

Note. Here and in Table 2: *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared to the control.

TABLE 2. Structural and Functional Characteristics of Erythrocyte Membranes ($M\pm m$)

Parameter	Control	Time after PF injection, h		
		24	72	168
F_E/F_M (334)	0.53±0.03	0.47±0.06	0.47±0.06	0.64±0.03***
F_E/F_M (282)	0.91±0.09	0.71±0.03***	0.53±0.04***	0.56±0.06***
F_0-F/F_0	0.111±0.011	0.122±0.036	0.121±0.008	0.065±0.003**
F_{372}/F_{393} (334)	1.21±0.05	0.99±0.04**	0.96±0.08**	1.17±0.05
F_{372}/F_{393} (282)	1.79±0.06	1.77±0.07	1.77±0.08	1.76±0.06

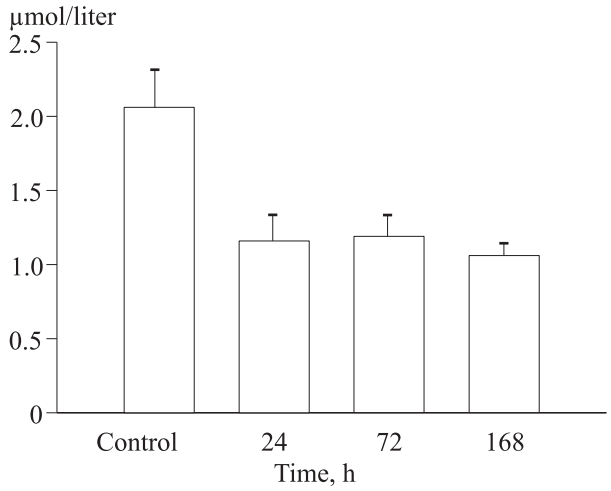

Fig. 2. Extraerythrocytic hemoglobin concentration in the plasma from control rats and animals receiving perftoran.

ous studies showed that EEH plays a role in free radical oxidation. The interaction between EEH and H_2O_2 (biological Fenton reagent) results in production of the hydroxyl radical ($^{\bullet}OH$) and ferryl radical (Hb-Fe=O). These compounds act as strong activators of LPO [1,2]. The observed changes aggravate metabolic disturbances, which manifests in uncontrolled secondary activation of LPO, increase in the severity of hypoxia, and activation of cellular proteolysis (*e.g.*, erythrocytes). The increase in free hemoglobin concentration in the blood aggravates microcirculatory disturbances and causes thrombocytopenia. Hemoglobin competitively interacts with serotonin receptors on platelets and vascular smooth muscles in the microcirculatory bed and displaces serotonin. It results in enhanced lysis of erythrocytes, vasospasm in the microcirculatory system, and development and/or aggravation of circulatory hypoxia [4].

Our results indicate that single injection of PF moderately activates LPO in erythrocyte membranes, increases stability of erythrocyte membranes (decrease in blood EEH concentration), and optimizes structural and functional characteristics of erythrocyte membranes. This manifests in increased structuredness of the annular lipid zone and decreased polarity of the lipid bilayer.

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