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Activity of membrane-bound NADH-methemoglobin reductase and physical state of lipids in erythrocyte membranes

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Abstract

The activity of membrane-bound NADH-methemoglobin reductase in erythrocytes and the physical state of lipids in erythrocyte membranes under oxidative stress in cells were studied. A decrease of the activity of membrane-bound NADH-methemoglobin reductase and a change of physical state of the lipid bilayer of membranes under oxidative stress were found in erythrocytes in vivo and in vitro.

Keywords: NADH-methemoglobin reductase; Erythrocytes; Oxidative stress

1. Introduction

Erythrocytes have a powerful antioxidant protection system. However, at a high concentration of free radicals or insufficiency of the primary antioxidant protection, the oxidative damage of the erythrocyte membrane components leads to loss of the ability of erythrocytes to transfer O_2 and CO_2, and brings about cell hemolysis. Oxidant-induced hemolysis can also take place in other cases such as during physiological aging of erythrocytes.

In erythrocytes under oxidative stress, there is a considerable rise in the level of methemoglobin, which is known to be incapable of reversible oxygen binding. Human erythrocytes normally contain ~1–3% methemoglobin. For converting methemoglobin into oxyhemoglobin, there are two enzymatic systems in erythrocytes, one of which is related to glycolysis and the other is associated with the pentose phosphate pathway [1,2]. Correspondingly, two types of enzymes function in erythrocytes: NADH-methemoglobin reductase and NADPH-methemoglobin reductase, both having cytoplasmic and membrane-bound forms. It is generally assumed that the reduction of oxidized hemoglobin with the participation of membrane-bound NADH-methemoglobin reductase is of the greatest physiological significance [3].

The purpose of this work is to elucidate the dependence of the activity of membrane-bound NADH-methemoglobin reductase in erythrocytes on the physical state of the membranes' lipid bilayer.

2. Materials and methods

Experiments were carried out on human erythrocytes, which had been exposed to oxidants in vitro; on a fraction of "young" and "aged" erythrocytes, on erythrocytes of patients with ischemic heart disease (IHD) and on erythrocytes of children with different blood levels of lead. The erythrocytes were prepared by centrifugation of cells suspended in a density gradient of Percoll [4]. The activity of membrane-bound NADH-methemoglobin reductase was estimated by assessing the spectrophotometrically rate of NADH-oxidation (Specord M 40 spectrophotometer, Germany) [5]. The physical state of membrane lipids was determined by assessing the fluorescence of the lipophilic probe 1.6-diphenyl-1.3.5-hexatriene (DPH), incorporated in ghosts of erythrocytes (LSF 222 luminescence spectrophotometer, SOLAR, Belarus). The binding of the fluorescent probe, DPH, to the erythrocyte membranes was measured as described in Ref. [6].

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3. Results

It was found that, in membranes isolated from human erythrocytes, incubation in medium containing 0.1–1 mM of phenylhydrazine, 0.1–1 mM *tert*-butyl hydroperoxy (*t*-BHP) or 0.5–3 mM H_2O_2 resulted in a reduction of the activity of membrane-bound NADH-methemoglobin reductase by 30–60% in comparison with control. This response was dose-dependent. The decrease of the intensity and the increase of the degree of polarization of fluorescence DPH provide evidence for changes in microviscosity of lipids (Fig. 1). This was dependent on the concentration of oxidants. It was found that the activity of membrane-bound NADH-methemoglobin reductase was 40–50% lower in 'aged erythrocytes' in comparison to 'young erythrocytes' (Table 1).

The patient group used to study the activity of the membrane-bound NADH-methemoglobin reductase were Class IV IHD patients (Canadian Classification, based on degree of stable angina). The activity of this enzyme was reduced by 20–30% in erythrocytes of patients in comparison to controls. The degree of polarization of fluorescent DPH incorporated in membranes of the Class IV IHD patients was also reduced by 20–25% in comparison to controls.

The results suggest that the activity of membrane-bound NADH-methemoglobin reductase is inhibited in parallel with a change in the physical state of the lipid bilayer of membranes under oxidative stress in erythrocytes. This model system (the incubation of erythrocytes in buffer containing oxidants at sublytic concentrations in vitro) is representative of the conditions experienced by cells in the process of aging, where there is an increase in the active forms of oxygen ('aged erythrocytes'), in IHD patients and in children with an increased blood lead level (≥ 0.1 mg/l),

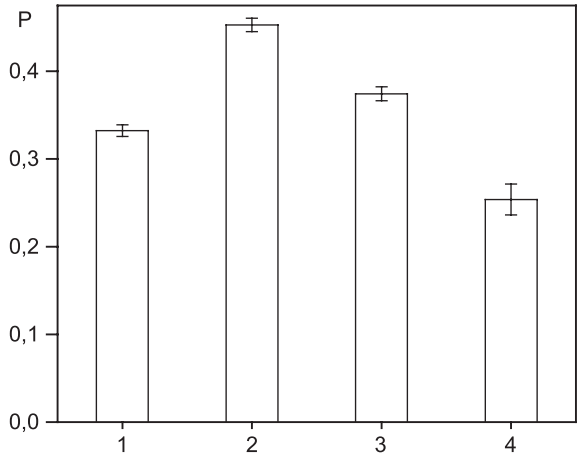

Fig. 1. The change of the degree polarization of the fluorescence of the lipophilic probe DPH, incorporated in ghosts of erythrocytes under oxidative stress in vivo and in vitro. (1) Control, (2) after incubation of cells with 1 mM of *t*-BHP, (3) after incubation of cells with 3 mM of H_2O_2, (4) of the patients with IHD (IV functional class).

Table 1
Activity of membrane-bound NADH-methemoglobin reductase in erythrocytes

Membrane were isolated from erythrocytes	A(rel. un.)	% of inhibition of activity in comparison with control
Volunteers (control)	31.61 ± 1.37 $n=28$	
After incubation with 1 mM of phenylhydrazine	12.87 ± 2.49 $n=8$	59
After incubation with 1 mM of *t*-BHP	16.75 ± 2.24 $n=5$	47
After incubation with 3 mM of H_2O_2	21.75 ± 1.42 $n=5$	31
After separation in density gradient of Percoll: fraction of "young" cells (control)	37.82 ± 2.01 $n=5$	
Fraction of "aged" cells	19.78 ± 2.27 $n=5$	48
Of the patients with IHD (IV functional class)	23.89 ± 1.58 $n=9$	24
Children with different level of blood lead: concentration of lead in blood <0.1 mg/l (control)	29.39 ± 1.16 $n=26$	
Concentration of lead in blood ≥ 0.1 mg/l	22.83 ± 1.26 $n=10$	22

all of which are associated with an increase in the active form of oxygen.

The correlation analysis shows that there is a connection between the activity of membrane-bound NADH-methemoglobin reductase and level of accumulation of products of peroxide oxidation of lipids in erythrocyte membranes under oxidative stress.

The inhibition of activity of membrane-bound NADH-methemoglobin reductase in human erythrocytes under oxidative stress depends on a physicochemical state of membrane lipids.

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