Changes in Electrokinetic Properties of Erythrocytes under the Influence of Pentoxifylline and New Hemorheologically Active Substances

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 2, pp. 174-176, February, 2012
Original article submitted November 22, 2010

We studied the impact of compounds RU-1202 and SUM-55 on electrophoretic mobility of "young" and "old" erythrocytes fractionated in a density gradient. The test compounds are shown to increase electrophoretic mobility of erythrocytes, compound SUM-55 being superior to the reference drug pentoxifylline.

Key Words: *electrophoretic mobility; fractionation of erythrocytes; SUM-55; RU-1202; pentoxifylline*

Electrokinetic potential of erythrocytesis is an important parameter responsible for suspension stability of the blood [4]. The surface electric charge of erythrocytes is mainly determined by dissociation of three functional groups binding to different membrane sites: sialic acids, certain types of carboxylic groups and weak basic amino groups [12]. Changed negative charge of erythrocytes and, consequently, their altered electrophoretic mobility (EPM) is observed in a variety of diseases and can be due to damage of cell structures or changes in the environment or result from erythrocyte aging [1,3,5,6,10]. It is now known that drugs that modulate intracellular processes in erythrocytes are capable to change the amount of charge on the cell membrane [2].

In our previous studies on pathological models and *in vitro* on erythrocytes with altered mechanical properties we revealed a pronounced effect of the compounds RU-1202 and SUM-55 on erythrocyte membrane surface charge manifesting in enhanced fluorescence of positively charged fluorescent probe DSM [9].

Here we studied the impact of compounds RU-1202 and SUM-55 on EPM of erythrocytes fractionated into "young" and "old" cells in a density gradient.

MATERIALS AND METHODS

We studied compound SUM-55 (1,3-dimethyl-7-(2-hydroxy-3-1-piperidinopropil)-8-phenylxanthine amine hydrochloride) [7], compound RU-1202 (9-(2-diethylaminoethyl)-2-(4-fluorophenyl) imidazo[1,2-a]benzimidazole dihydrochloride) [8], and pentoxifylline, a hemorheologic agent (Aventis).

Experiments were performed on 50 outbred albino male rats weighing 270-300 g. The animals were kept under vivarium conditions at 22-24°C, humidity 40-50%, and natural illumination regime on a standard diet (GOST R 50258-92). The study was carried out in accordance with the rules of laboratory practice in conducting preclinical studies in the Russian Federation (GOST3 51000.3-96 and 1000.4-96), as well as the rules and international recommendations of European Convention for Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes,

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0007-4888/12/1532-0209

Council of Europe (ETS 123). Blood samples were taken from the abdominal aorta of anesthetized animals and stabilized with 3.8% sodium citrate (9:1).

Whole blood erythrocytes were divided into "young" and "old" cells as described previously [11]. The test substances were added to erythrocyte suspensions in a final concentration of 10^{-4} mol/liter immediately before incubation. Equivalent volume of warm saline (37°C) was added to the control samples. After incubation, EPM was determined in a microchamber with flat silver chloride electrodes using standard phosphate buffer (pH 7.38, 300 mOsm) as the electrophoretic medium. Erythrocyte potentials (ζ) were calculated using the Smoluchowski equation using measured EPM of cells and known viscosity (η) and dielectric constant of the medium (D).

The numerical data were statistically processed using Microsoft Excel.

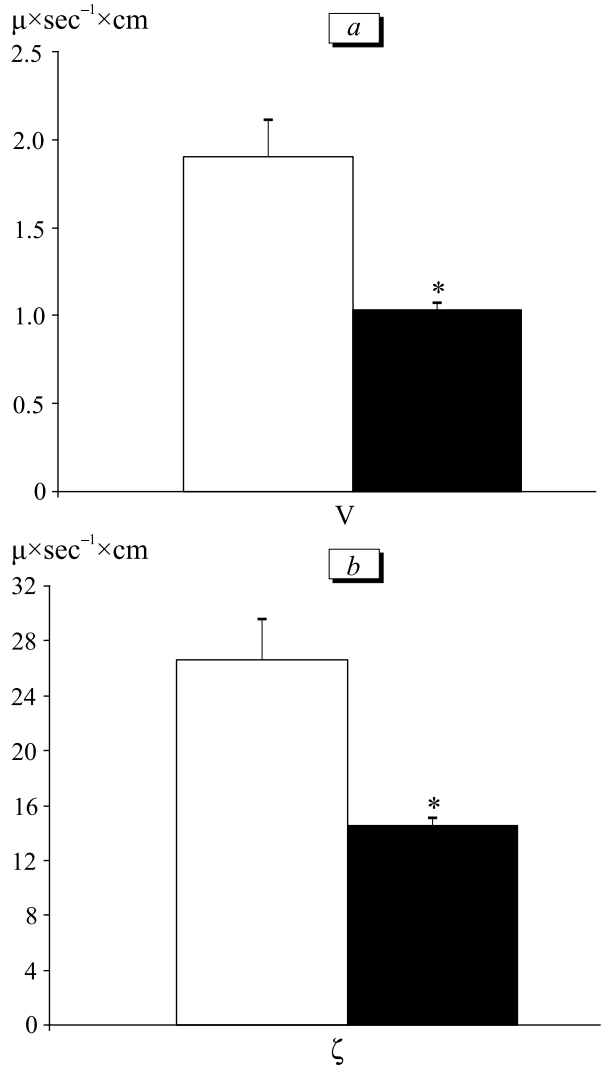

Fig. 1. EPM (V, *a*) and ζ potential (*b*) of fractionated young (light bars) and old (dark bars) erythrocytes. *$p<0.05$

RESULTS

EPM of "old" erythrocytes was reduced compared to that of "young" erythrocytes. Significant reduction in electrokinetic potential (ζ potential) was also observed (Fig. 1). Reduced erythrocyte membrane potential results in its destruction because of a decrease in field strength on the membrane decreases. This disturbs the interaction between components of erythrocyte membranes (phospholipids, esters of cholesterol, triglycerides) and leads to intensification of lipid peroxidation processes and impairs oxygen-transporting functions of erythrocytes.

The test compounds and reference drug had no significant effects on electrophoretic mobility and ζ potential of "young" erythrocytes. However, the opposite situation was observed in a fraction of "old" erythrocytes. Compound SUM-55 increased erythrocyte EPM by 48%, which surpassed pentoxifylline activity by 16%. Compound RU-1202 was inferior to pentoxifylline and increased erythrocyte EPM by 14%. However, these data were insignificant.

The increase in EPM was accompanied by an increase in ζ potential. Compound SUM-55 increased this parameter by 48%, RU-1202 by 14%, and pentoxifylline by 31%.

The study showed that compounds SUM-55, RU-1202, and pentoxifylline increase EPM of erythrocytes, which in turn confirms their capacity to increase surface charge of erythrocytes. Since the power characteristics of the field, pH, ionic strength, dielectric constant, viscosity, and temperature of the medium remained constant during the experiment, increased EPM of erythrocytes under the influence of the studied compounds can be explained by their influence on the structure of erythrocyte membranes. This assumption is confirmed by the data on the effect of the test compounds on microviscosity of erythrocyte membranes. Compounds RU-1202 and SUM-55 in a wide concentration range of 10^{-4}-10^{-6} mol/liter significantly reduced the rate of DSP-6 fluorescence anisotropy

TABLE 1. Effect of the Compounds SUM-55, RU-1202, and Pentoxifylline on EPM (V) and ζ-Potential of Erythrocytes (*M*±*m*)

Group	V, μ×sec⁻¹×cm	ζ, mV
Control (old erythrocytes)	1.03±0.04	14.55±0.60
Compound SUM-55	1.53±0.08*	21.52±1.90*
Compound RU-1202	1.18±0.13	16.57±1.10
Pentoxifylline	1.36±0.08*	19.1±0.9*

Note. *$p<0.05$ relative to the control.

in erythrocyte suspension which points to decreased microviscosity of cell membrane lipids.

Thus, these findings suggest that membranotropic activity of compounds SUM-55 and RU-1202 underlies their mechanism of action.

The work was supported by Russian Foundation for Basic Research (09-04-90783_mob_st).

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