OSMOTIC RESISTANCE AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND GLUTATHIONE REDUCTASE ACTIVITY OF ERYTHROCYTES IN PATIENTS WITH LEUKEMIAS AND ANEMIAS

I. S. Luganova and I. F. Seits

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The osmotic resistance of the erythrocytes is lowered in patients with chronic myeloid and lymphatic leukemia, acute leukemia, polycythemia, Marchiafava disease, and hemoplastic anemias. In chronic lymphatic and acute leukemia the glucose-6-phosphate dehydrogenase and glutathione reductase activity is lowered, in Marchiafava disease it is raised, and in polycythemia and the hemoplastic anemias it is unchanged. In chronic myeloid leukemia the activity of glucose-6-phosphate dehydrogenase only is raised.

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One cause of the development of anemia in patients with leukemias may perhaps be the chemical-structural and functional defectiveness of the erythrocytes. The physiological normality of the erythrocytes and their resistance to hypotonic solutions and to certain drugs are determined by the ability of these cells to split hydrogen peroxide formed in the course of their metabolic activity [1-3].

The whole system of enzymes and reactions responsible for this aspect of the structural integrity of the erythrocytes can be represented schematically as follows: g-6-p* + NADP $\xrightarrow{\text{g-6-p dehydrogenase}}$ 6-phosphogluconate + $NADP \cdot H_2$; GSSG + $NADP \cdot H_2$ $\xrightarrow{\text{glutathione reductase}}$ GSH + NADP; H_2O_2 + 2GSH $\xrightarrow{\text{glutathione peroxidase}}$ $2H_2O$ + GSSG. Omission of one of the links of this chain of reactions leads to disturbance of the physiological integrity of the erythrocytes.

The object of the present investigation was to study activity of g-6-p dehydrogenase, 6-phosphogluconate dehydrogenase, and glutathione reductase, on the one hand, and the osmotic resistance of the erythrocytes of patients with various forms of leukemias on the other.

EXPERIMENTAL METHOD

Erythrocytes were obtained from venous blood stabilized with heparin (10 units/ml blood). The erythrocytes were separated from leukocytes and platelets in gelatin–citrate solution followed by washing three times in physiological saline. Hemolysates were prepared from densely centrifuged erythrocytes by addition of 20 volumes of cold distilled water. Activity of g-6-p dehydrogenase, 6-phosphogluconate dehydrogenase, and glutathione reductase was determined spectrophotometrically by the change in absorption of light at 340 mμ at room temperature (details are given in Table 1). The osmotic resistance of the erythrocytes was determined by counting those remaining after exposure for 1 h to 0.48% NaCl solution at room temperature. The number of hemolyzed erythrocytes was expressed as a percentage of the number of erythrocytes in 1% NaCl solution.

*The following abbreviations are adopted in this paper: g-6-p glucose-6-phosphate, GSH reduced glutathione, GSSG oxidized glutathione.

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TABLE 1. Osmotic Resistance and Activity of Glucose-6-Phosphate and 6-Phosphogluconate Dehydrogenases and of Glutathione Reductase in Normal and Pathological Human Erythrocytes, M ± m

Subjects from whom erythrocytes obtained	No. of experiments	% of erythrocytes hemolyzed in 0.48% NaCl	Activity of enzymes (in μmoles substrate/min/ml erythrocytes)		
			g-6-p dehydrogenase	6-phosphogluconate dehydrogenase	glutathione reductase
Healthy	23	11.9 ± 0.76	1.26 ± 0.03	0.23 ±0.01	0.23 ± 0.01
Patients with chronic myeloid leukemia	13	42.2 ± 2.64 P < 0.01	1.52 ± 0.09 P < 0.01	0.28 ± 0.02 P < 0.05	0.21 ± 0.02 P > 0.2
Patients with chronic lymphatic leukemia	21	46.5 ±2.19 P< 0.001	0.88 ± 0.04 P < 0.001	0.24 ±0.02 P > 0.5	0.16 ± 0.03 P < 0.005
Patients with acute leukemia	11	49.3 ±3.90 P < 0.001	0.89 ± 0.01 P < 0.001	0.22 ± 0.02 P <0.2	0.16 ± 0.02 P < 0.001
Patients with polycythemia	17	23.3 ± 4.30 P < 0.01	1.30 ±0.11 P > 0.5	0.26 ±0.02 P > 0.2	0.21 ± 0.03 P > 0.2
Patients with Marchiafava disease	10	32.7 ± 5.31 P < 0.001	2.17 ± 0.14 P < 0.001	0.25 ±0.02 P >0.5	0.34 ± 0.03 P < 0.001
Patients with hypoplastic anemia	8	22.6 ±5.01 P < 0.05	1.20 ± 0.09 P > 0.5	0.20 ± 0.02 P > 0.1	0.19 ± 0.03 P > 0.2

Note. Experimental samples, 3 ml in volume contained (in μmoles) for determination of g-6-p dehydrogenase: g-6-p 2; $MgCl_2$ 20; tris buffer, pH 7.4, 125; NADP 1; test hemolysate (1:20) 0.1 ml; for determination of 6-phosphogluconate dehydrogenase the composition of the samples was the same except that g-6-p was replaced by 3 μmoles 6-phosphogluconate; for determination of glutathione reductase: GSSG 10; K-phosphate buffer, pH 7.5, 250; $NADP \cdot H_2$ 0.5; test hemolysate 0.1 ml.

EXPERIMENTAL RESULTS

The results of the study of osmotic resistance and activity of g-6-p and 6-phosphogluconate dehydrogenase and glutathione reductase in normal and pathological erythrocytes are summarized in Table 1.

It is clear from Table 1 that the number of hemolyzed erythrocytes from leukemia patients was about four times greater than normal, twice greater for polycythemia and hypoplastic anemia, and three times greater for Marchiafava disease. A decrease in activity of g-6-p dehydrogenase and glutathione reductase might have been expected in the erythrocytes of patients with leukemia, because of the low osmotic resistance of these cells. In fact, the erythrocytes suspension obtained from patients with chronic myeloid leukemia showed higher activity of g-6-p dehydrogenase and 6-phosphogluconate dehydrogenase than normally, presumably because of the presence of a high proportion of reticulocytes in the blood of these patients (from 2 to 10% compared with the normal 0.8%), and these have high indices of activity of many enzymes. The increased g-6-p dehydrogenase and glutathione reductase activity in the erythrocytes in Marchiafava disease may also be explained by an increase in the reticulocyte count.

A statistically significant decrease in activity of g-6-p dehydrogenase and glutathione reductase was found in the erythrocytes of patients with chronic lymphatic and acute leukemia, presumably on account of their particularly high sensitivity to the hemolytic action of hypotonic solutions. The activity of all three enzymes was unchanged in the erythrocytes of patients with polycythemia and the hypoplastic anemias.

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