Typical Changes of Reversible Erythrocyte Aggregation in Various Pathological Processes

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Examinations of patients with malignant tumors of different location (lung cancer, stomach, colorectal cancer, and head and neck tumors), patients with paranoid schizophrenia and neurotic disorders revealed enhanced reversible erythrocyte aggregation. It was found that enhancement of erythrocyte aggregation is associated with structural modification of their membrane and appearance of transformed cells (echinocytes, stomatocytes, *etc.*). The phenomenon of enhanced reversible erythrocyte aggregation in somatic and mental diseases can be regarded as a typical pathological process.

Key Words: *reversible erythrocyte aggregation; pathological process; typical reaction*

The search for specific criteria of structural changes in cells in various pathologies is associated with many difficulties and errors determined by continued specialization of medical science without proper attention to integrative evaluation of the structural, metabolic, and functional disorders at the cellular level from the viewpoint of the whole organism. It is however obvious that all cell systems normally functioning in accordance with their own laws can get out of the control of regulating systems under pathological conditions and exhibit universal reactions to pathogenic factors. In order to identify typical changes in cell systems in various pathologies we investigated reversible erythrocyte aggregation (EA), an integral parameter reflecting structural and functional state of these cells [11,14].

MATERIALS AND METHODS

The study was carried out in 130 patients with cancer and mental disorders (102 men and 28 women) aged 21-64 years. Thirty-six patients had lung cancer (stages III-IV), 11 had stomach cancer, 15 colorectal cancer, 19 head and neck tumors, 27 had paranoid schizophrenia, and 22 neurotic disorders (neurasthenia, adaptation disorders). None patients had concomitant diseases. The study was carried out before treatment. Control group consisted of 46 donors without somatic or mental diseases (34 men and 12 women) aged 20-52 years. Peripheral and venous blood for the analysis was collected after overnight fasting.

Reversible EA was evaluated by the photometric method based on the dependence of blood optical density on the degree of erythrocyte aggregation [11]. The minimum and maximum resistance of erythrocyte aggregates was evaluated by measuring the intensity of light passing through the blood sample. Spontaneous aggregation half-time (τ) was determined and the aggregation index reflecting the aggregation/disaggregation ratio and the integral EA coefficient were calculated [1].

Morphological characteristics of erythrocytes were studied by scanning electron microscopy. The samples were prepared as described previously [6] and examined under a PEM-200 electron microscope. A total of 1000 cells per preparation were counted using classifications proposed previously [5,6].

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Membrane preparations for evaluation of microviscosity of the lipid phase of erythrocyte membrane (EM) were prepared by hypoosmotic hemolysis and incubated with lipotropic fluorescent probe pyrene [2]. The degree of pyrene eximerization equal to eximer/monomer fluorescence intensity maxima (λ=470 nm and λ=370 nm, respectively) ratio characterizing mobility of lipid carbohydrate chains and hydrophobic volume of EM [2] was estimated. Spectral characteristics of pyrene interactions with EM were recorded on a Hitachi-MPF4 spectrofluorometer at 340 nm excitation wavelength.

Normal distribution of the analyzed parameters was verified using Kolmogorov—Smirnov test; the equality of selected means was verified using Student's *t* test (for normal distribution) and Mann—Whitney's *U* test (for non-normal distribution). Analysis of correlations was carried out by estimating Spearman's coefficient.

RESULTS

Clear-cut signs of disordered microrheological status of erythrocytes were detected in patients with somatic and mental diseases. The mean values of minimum resistance of erythrocyte aggregates, aggregation index and coefficient, and spontaneous EA rate were significantly increased in patients with paranoid schizophrenia and neurotic disorders (τ significantly decreased in patients in comparison with donors, Table 1). Similar disorders of erythrocyte aggregation were detected in patients with malignant tumors. The most pronounced changes in reversible EA were observed in patients with lung cancer: the minimum and maximum resistance of aggregates, aggregation index and coefficient surpassed those in donors by 1.7, 1,5, 4.6, and 8.7 times, respectively. The mean τ in this group was 3.2-fold below the normal (Table 1). Minimum deviations of the studied parameters of erythrocyte aggregation were detected in patients with stomach cancer. Previously we detected the phenomenon of reversible AE stimulation in patients with insulin-dependent diabetes mellitus, acute pneumonia, chronic bronchitis, peptic ulcer, autoimmune thyroiditis, atherosclerotic involvement of the lower limb arteries, *etc.* [7-10].

It is known that erythrocyte aggregation capacity is determined, apart from plasma factors, by physicochemical characteristics of EM, its plasticity, and den-

TABLE 1. Parameters of Reversible EA (% of Values in Donors) in Patients with Cancer and Mental Disorders ($X \pm m$)

Parameter	Lung cancer	Stomach cancer	Colorectal cancer	Head and neck tumors	Paranoid schizophrenia	Neurotic disorders
Mechanical resistance of aggregates						
minimum	113.3±5.0***	76.8±10.2	86.8±10.0	92.0±5.3	132.8±6.3**	108.3±4.7***
maximum	106.5±3.7***	73.8±6.3	75.7±6.9	87.3±6.9	99.4±2.0	88.9±3.3
τ	16.0±1.1*	22.5±4.6*	18.7±1.3*	20.1±2.8*	22.4±1.9*	31.6±2.6**
Aggregation coefficient	321.7±42.3*	122.7±14.3***	135.4±18.5**	192.0±36.8*	462.8±93.7*	246.3±42.0*
Aggregation index	188.9±18.9*	118.8±14.8***	116.0±12.7***	165.2±37.7**	211.8±13.0*	145.0±15.7**

Note. *p<0.001, **p0.01, ***p<0.05 compared to donors.

TABLE 2. Correlations (Spearman Coefficient of Correlations) between Reversible AE and Parameters Characterizing EM Microviscosity and Erythrocyte Morphology in Patients with Mental Disorders

Parameters of reversible erythrocyte aggregation	Degree of pyrene eximerization, arb. units	Crest discocytes	Echinocytes	Stomatocytes	Spherocytes	"Let-out ball" erythrocytes
Mechanical resistance of aggregates						
minimum	-0.84*	0.51***	0.68*	0.76*	0.54**	0.59**
maximum	-0.52***	0.09	0.32	0.37	0.12	0.23
τ	0.70*	-0.51***	-0.71*	-0.78*	-0.62**	-0.54**
Aggregation coefficient	-0.75*	0.46	0.66**	0.75*	0.56**	0.54**
Aggregation index	-0.67**	0.43	0.68*	0.71*	0.52***	0.45

Note. Significance of correlations: *p<0.001, **p<0.01, ***p<0.01.

sity of negative charge on the cell surface [12]. Increase of reversible EA can be caused by changes in the qualitative composition of EM phospholipids, disorders in transmembrane distribution of individual phospholipid molecules [3,4]. For example, A. Othmane *et al.* [15] observed an increase in reversible EA after modification of phospholipid composition in the outer EM leaflet by disordering phospholipid transmembrane asymmetry via an increase in intracellular Ca^{2+} content and incorporation of lysoderivatives into the membrane. Blockade of the mechanism maintaining the initial phospholipid composition asymmetry leads to redistribution of phospholipids, which promotes transformation of athrombogenic cell surface into thrombogenic [4].

Our study also confirmed the role of structural disorders of EM in the mechanisms stimulating reversible EA in various pathologies. We previously detected pronounced changes in protein and lipid composition of EM, changes in protein-lipid interactions, modulation of activities of the membrane cation-transporting systems, disorganization of surface architectonics of erythrocytes in patients with tumors and mental diseases [9,10]. In the present study we showed that enhanced erythrocyte aggregability in various pathologies is associated with increased microviscosity of the cell membrane lipid bilayer (Table 2). We showed that intensification of EA in the examined patients was caused by accumulation of crest discocytes, stomatocytes, spherocytes, "let-out ball" erythrocytes, and echinocytes in the erythrocyte population, which is in line with published data on the role of erythrocyte transformations in the genesis of their increased aggregability. For example, the appearance of numerous echinocytes in the circulation increases blood viscosity and modulates aggregation characteristics [14]. A. M. Chernukh *et al.* [13] hypothesized that the appearance of surface processes and wrinkles of the erythrocyte membrane increased the area of contacting surfaces, thus triggering aggregate formation.

Hence, increased reversible EA in various pathologies should be regarded as a typical pathological process realized at the level of the peripheral component of the erythron and having no specific nosological and etiological characteristics. From a general biological viewpoint, stereotypical structural and functional changes in erythrocytes in mental and somatic diseases suggest lower variability of biological response to pathogenic factors and allow us to conclude that the reaction of the circulating erythrocyte pool is universal in all pathologies. This assumption on the basic mechanisms and common regularities of reactions of the peripheral erythron, a highly sensitive test system of the internal media of the body, in various diseases opens new vistas for understanding the common biological laws regulating the development of pathological processes.

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