EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Structural Composition of Blood Erythrocytes in Children with Nephropathies

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Study of the structural characteristics of peripheral blood erythrocytes in the pathogenesis of nephropathies in children by means of automated recording of integral osmotic erythrograms and analysis of differential curves of hypoosmotic hemolysis showed decreased osmotic resistance of erythrocytes, which was most pronounced during renal dysfunction. A molecular model of possible modification of the erythrocyte membrane in the pathogenesis of nephropathies is proposed.

Key Words: *erythrogram; osmotic resistance; nephropathy*

Recent progress in nephrology is largely due to introduction of modern diagnostic and therapeutic methods into clinical practice [5]. However the mechanisms underlying the development of renal diseases remain poorly studied [3]. Therefore study of the relationship between structural characteristics of peripheral blood erythrocytes and different forms of nephropathy at the molecular level is today the most important approach in medical biochemistry and biophysics.

We chose two variants of glomerulonephritis (GN) for our study: with normal and impaired renal function and the hemolytic uremic syndrome (HUS) during acute renal failure (ARF) and recovery of renal function. Characteristic signs of renal failure are disorders in water and salt homeostasis and plasma acid base equilibrium, as well as accumulation of intermediate products of protein metabolism (urea, residual nitrogen, creatinine, uric acid, *etc.*) [4,6]. Changes in the erythron system are clinically diagnosed in renal diseases: erythropoiesis is disordered and erythrocyte degradation is activated [4,7].

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MATERIALS AND METHODS

Structural characteristics of peripheral blood erythrocytes were studied in children aged 8-12 years hospitalized at the Voronezh regional pediatric clinical hospital. Thirteen children with GN (in 10 of these the disease was aggravated by chronic renal failure, CRF) and 5 with HUS were examined before (with ARF) and after treatment.

In order to detect latent structural transformations in erythrocytes, the kinetics of hypoosmotic (0.55% NaCl) hemolysis in erythrocyte suspension was studied (photometry of hemolysis in cells with different stability). Erythrocytes were washed 3 times with 0.9% NaCl at 3000 rpm.

Integral hypoosmotic erythrograms were recorded on a PEC-56M photocolorimeter with V7-20 voltmeter and LKD4-003 recorder [7]. Erythrocytes were put in thermostated cuvettes (20×40×10 mm, working volume 4 ml) and light transmission (T, %) in the suspension was measured at λ=490 nm and constant pH 6.8 after agitation.

The distribution (dispersion) of structural modifications in the erythrocyte population was evaluated by differential curves of hypoosmotic hemolysis plotted as described previously [1].

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The integral and differentiation erythrograms were analyzed by the following parameters: number of hemolyzed erythrocytes (G, % of control, *i.e.* donor blood), constant of maximum hemolysis rate at the global maximum (K_{max}, arb. units), half-width of the left and right parts of the differentiation curve, and latency. The results were processed by methods of variation statistics using parametrical tests.

RESULTS

Measurements revealed latent structural abnormalities in discocyte membranes and cytoskeleton structures (Fig. 1). The dynamics of the structural status of the peripheral erythron component correlated with phasic changes in the parameters reflecting activity of the pathological process (proteinuria, hematuria, renal function, increased erythrocyte sedimentation rate, *etc.*).

Normally, the decrease in osmolarity of the incubation medium to a threshold values of osmotic resistance of erythrocytes leads to hemolysis of 80-92% cells (Fig. 1, curves *1* and *3*). Analysis of integral curves showed that the most pronounced decrease in osmotic resistance of erythrocytes was observed in patients of both groups during the period of renal dysfunction (ARF and CRF).

The observed changes in the structure and function of peripheral blood erythrocytes probably reflect a nonspecific response to increased plasma concentrations of hemolytically active metabolites (urea, creatinine, *etc.*).

Hence, the development of renal failure in GN and HUS is associated with structural modification of membranes and cytoskeletal complexes in primarily old erythrocytes, which determines decreased osmotic resistance of erythrocyte population (G=18-92%).

Quantitative evaluation of structural modifications and their distribution in erythrocyte subpopulations was carried out using differentiation erythrograms; analysis of these curves revealed structural heterogeneity of erythrocyte population, which correlated with the severity of the disease.

Significant differences in the composition of erythrocyte population in renal failure were detected (Fig. 2, curves *1* and *3*). Similar position and height of the global peaks (K_{max}=4.5 arb. units) in patients with GN and HUS during renal dysfunction attest to high degree of cell homogeneity. However different half-widths of the left and right parts of differentiation curves indicate different contribution of individual discocyte subpopulations to the total osmotic resistance of erythrocytes. The differential curves in patients with HUS and ARF were asymmetric (Fig. 2, curve *1*) because of high content of low-resistant cells (elevated left part of the curve), while erythrograms of patients with GN with CRF were characterized by increased half-width of the right wing indicating high content of highly resistant cells in the erythrocyte population. The appearance of several peaks on the differentiation curves reflected high structural heterogeneity of erythrocyte subpopulations (Fig. 2, curves *1* and *3*). The detected distributions can be explained by peculiarities of organism's adaptation to acute and chronic nephropathy. The intensity and duration of the pathological process seem to determine the direction of the compensatory response the erythron to a particular pathology.

For evaluation of the relationship between structural characteristics of peripheral blood erythrocytes and severity of nephropathy we studied osmotic resis-

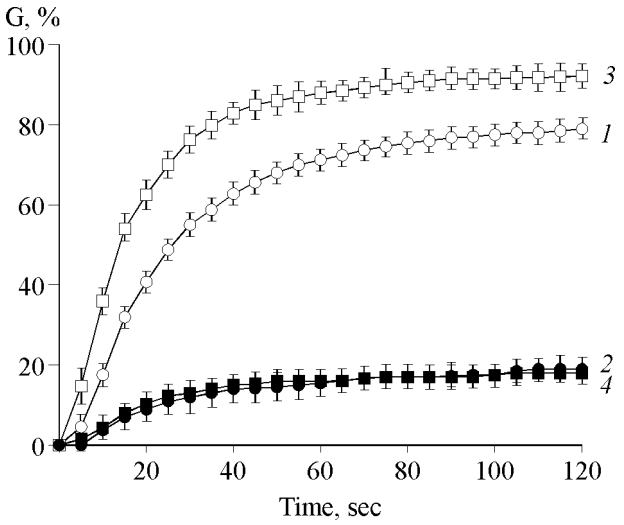

Fig. 1. Kinetics of hypoosmotic hemolysis of erythrocytes in patients with nephropathies. G: erythrocyte resistance index. Here and in Fig. 2: *1*, *2*) hemolytic uremic syndrome during acute renal failure and after therapy, respectively; *3*, *4*) glomerulonephritis with chronic failure and without renal dysfunction, respectively.

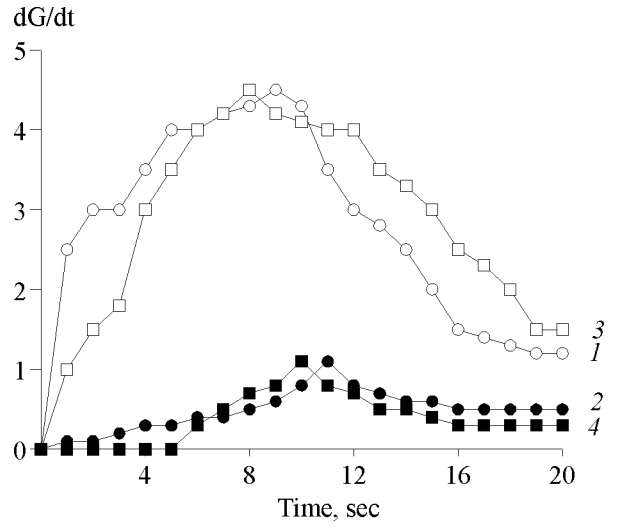

Fig. 2. Differentiation erythrograms of hypoosmotic hemolysis of blood erythrocytes in patients with nephropathies. dG/dT: increase in the number of degraded erythrocytes per time unit.

tance of erythrocytes in patients with GN without CRF and with HUS during remission.

Comparison of the experimental findings revealed a marked decrease in the number of cells hemolyzed in hypotonic medium (G approximately 20%) in patients with GN compared to the initial value (Fig. 1, curve *1*) and in HUS patients compared to patients with GN without CRF (Fig. 1, curve *2*). These results indicate relative normalization of structural characteristics of modified erythrocytes or replacement of abnormal erythrocytes with native cells. The detected relationship confirms a direct relationship between the content of hemolytically active agents (endogenous toxic load) and the number of structural modifications in discocyte membrane and submembrane complexes.

The population composition of the peripheral elements of the erythron in the patients can be evaluated by the shape and position of differentiation erythrograms. The global maximum of the curve was shifted to the right in patients with GN and HUS (Fig. 2, curves *2* and *4*), which attested to increased content of highly resistant cells and increased heterogeneity of the erythrocyte population. The mean difference in K_{max} values in the patients with reduced and normal renal function was 27% (Fig. 2).

Hence, if renal function is restored or intact, the distribution of erythrocytes by osmotic resistance corresponds to the state of relative stabilization of the erythron structure and function and in general reflects the sensitivity and, hence, the degree of erythrocytes involvement in the pathological process.

The proportional relationship between the number of latent structural disorders (both in the erythrocyte membrane and cytoskeleton), and the age of the cell and metabolite content depends primarily on the molecular composition of the membrane. Depletion of the erythrocyte membrane with age increases plasmolemma permeability for toxins affecting the lipostromatin and spectrin-actin complexes. These changes reduce osmotic resistance of erythrocytes observed in our experiments. Structural degradation of cytoskeleton can also result from impaired protein-lipid interactions in the erythrocyte membrane.

According to current views, the pathogenesis of nephropathies is associated with activation of free radical oxidation against the background of impaired antioxidant defense [3,7]. We propose a molecular model of possible modification of the erythrocyte membrane in the pathogenesis of nephropathy as exemplified by the chemical reactions of its protein components with carbamide, a hemolytically active carbonyl metabolites.

The reaction with primary amines (lysine and arginine NH_3^+ groups and terminal α-NH_2 groups of protein molecules) is described by the following equation:

$$R—NH_2+O=C(NH_2)_2 \rightarrow R—N=C(NH_2)_2+H_2O.$$

Urea reacts with weak S nucleophiles (SH groups of cysteine):

$$R—SH+O=C(NH_2)_2 \rightarrow R—S—COH(NH_2)_2.$$

Activation and binding to strong nucleophiles (tyrosine OH groups *et al.*) are described by the following equation:

$$R—OH+O=C(NH_2)_2 \rightarrow R—O—COH\text{-}(NH_2)_2,$$

where R is the protein residue.

Complexation of carbamide with ionogenic groups in proteins reduces the length of α-helixes and β-sheets, increases the percentage of irregular structures, and induces the formation of additional hydrogen bonds between the urea amino groups and carbonyl groups of glutamic acid and asparagine, and hydroxyl groups of serine and threonine. Moreover, amide group of urea can attract intramolecular hydrogen bonds of the protein molecule determining the secondary structure of the polymer. Destruction of hydrogen bonds in the macromolecule changes the number of hydrophobic interactions responsible for spatial organization of the protein molecule [2].

These reactions of complexation between urea and membrane proteins change the surface charge of the protein globule, which decreases the strength and pattern of interactions between the lipid and protein molecules predominantly in the apolipid zone. This impairs the hydrophobic barrier of the lipid bilayer and increases erythrocyte membrane permeability for polar and charged ions, specifically for urea, ketones, aldehydes, and other metabolites.

Reactions of the urea with intracellular components (for example, with hemoglobin) initiate dissociation of tetramer to dimers and monomers because of a decrease in the number of bonds stabilizing the globule, which determines increased oncotic pressure and hemolysis of erythrocytes.

Hence, the development of different forms of nephropathies is associated with accumulation of metabolites reacting with erythrocytes and modifying their structure. These modifications reduce osmotic resistance and shorten the life span of circulating erythrocytes, *i. e.* cause hypoerythremia. Comparative analysis of *in vitro* findings and clinical parameters showed that treatment efficiency can be monitored by osmotic erythrogram parameters.

Our approach to the study of the initial structural changes in peripheral blood erythrocytes disclosed the relationship between the erythrocyte structure (resistance) and disease severity and type. Our method of

recording hypoosmotic erythrograms can be used for predicting treatment efficiency and disease outcome.

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