METHODS

Reduction of Erythrocyte Deformability in Rats with Cerebral Ischemia

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Erythrocyte deformability was studied by laser interferometry in rats with cerebral ischemia. Ninety minutes after ligation of both common carotid arteries the erythrocyte deformability coefficient decreased by 11±2% in experimental group in comparison with the baseline level and by 12% in comparison with sham-operated animals and remained 16% decreased after 4 days.

Key Words: *laser interferometry; shear flow; erythrocytes; cerebral ischemia*

Cerebral ischemia is the most prevalent disease of cerebral circulation. Changes in blood rheology (for example, erythrocyte deformability) in this condition remain poorly studied. Erythrocyte deformations are just a part of dynamic process based on incessant rotating movement of cell membrane round liquid cytoplasm, paralleled by vortex-like movement of hemoglobin solution. *In vivo* microphotography shows that even in large vessels red blood cells are constantly reoriented along the complex shear stress gradient; erythrocytes are elongated in vessels with $d>10$ μ, transforming into more or less regularly elongated ellipsoids, while in vessels with $d<10$ μ erythrocytes acquire an asymmetrical shape. Several methods for studies of deformation characteristics of erythrocytes are known by the present time: sucking of the membrane into a pipette, erythrocyte filtration through microfilters [2,4,12], and optical methods (interferometry [7,8]). The data on changes in erythrocyte deformability in cerebral ischemia are contradictory. Some authors detected changes in erythrocyte deformability in deficient blood supply to the brain [1], others observed no changes of this kind [6,11]. We used interferometry to compare erythrocyte deformability in control animals and animals with experimental cerebral ischemia.

MATERIALS AND METHODS

The design of our device is based on M. Bessis ectracytometer [8]. The interferometer consists of two coaxial cylindrical glasses forming a Couette cell. Diluted erythrocyte suspension is placed between the glasses. Rotation of the inner cylinder induced shear flow of the suspension in this gap. The distribution of flow velocity in radial direction can be considered linear and shear rate can be considerate constant.

$$g = \frac{2\pi RN}{d}$$

The $R=(R_1+R_2)/2$, R_1 and R_2 are radiuses of cylinders, d width of the gap between them and N rate of cylinder rotation in rpm. With known viscosity of the suspension η, the shear stress is approximately determined as follows: $\tau=\gamma\times\eta$.

Shear rate can change from 10 to 775 sec^{-1} in a step-wise manner, which corresponds to τ changes from 4 to 300 din/cm.

The optical part of the device consists of He-Ne laser; its ray is reflected by a mirror on the cylinder

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bottom and passes horizontally through the walls of cylinders, layer of erythrocyte suspension between them, and the lens and gives a diffraction picture, corresponding to the size and shape of an averaged erythrocyte in the videocamera, placed at a focus distance from the lens. Videoimage from the camera is digitalized and further processed by a computer.

The parameter of erythrocyte deformability was determined as

$$P = \frac{a-b}{a+b}$$

(Fig. 1), where a and b are the major and minor semiaxises of ellipses corresponding to the levels of similar intensity.

In order to estimate the error in measurement of the deformability parameter, its values were determined for each of 5 images for 3-5 levels of intensity for each value of shear rate. The mean quadratic deviation of measurements was 5-7% in all experiments.

Experiments were carried out on 19 Wistar rats (369±8 g). The animals were divided into 2 groups: control (n=9) and experimental (n=10). In experimental animals cerebral ischemia was induced by simultaneous ligature of both common carotid arteries under ether narcosis. In order to provide a reliable occlusion of the bloodflow, a second ligature was made on each artery and the vessel between the ligatures was crossed. Sham-operated animals served as controls; they were subjected to similar surgical manipulations under ether narcosis, but the common carotid arteries were left intact. Blood for analysis of erythrocyte deformability was collected from the jugular vein in all animals before the operation, during acute phase of cerebral ischemia (90 min after occlusion of common carotid arteries), and during chronic phase of ischemia (4 days after ligation of arteries). Blood (0.3 ml) was collected into tubes with 10 µl 7% EDTA, diluted with polyethylene oxide (molecular weight 5×10^6) prepared on 0.9% NaCl in 1:300 ratio. The suspension viscosity was 13 santipauses.

Erythrocyte deformability was measured by the diffractometer described above. The data were calculated and curves were plotted using Spiricon and Excel software.

The differences between experimental and control groups were evaluated using nonparametrical Mann—Whitney test; statistical data processing for each animal before and after the operation was carried out by Wilcoxon paired test using STATISTICA software.

RESULTS

Cerebral ischemia significantly lowered erythrocyte deformability coefficient in comparison with the control at shear rates from γ=38.7 sec^{-1} to 775.0 sec^{-1} as early as 90 min after ligation of the common carotid arteries; the coefficient remained low at shear rates of γ=17.4 sec^{-1} to 775 sec^{-1} under conditions of chronic ischemia 4 days after surgery (Fig. 2, *b*, *c*).

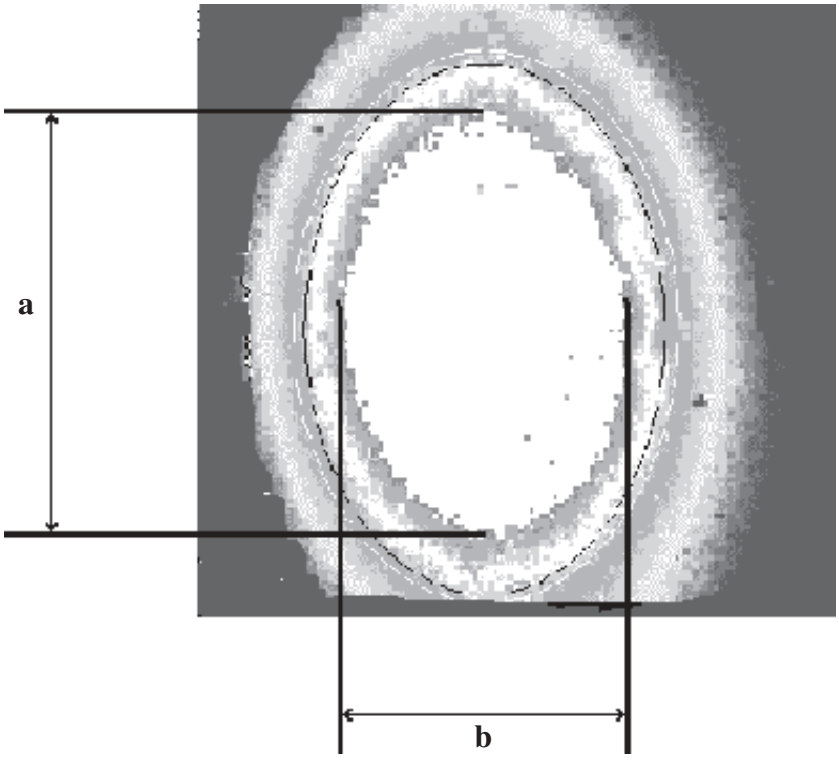

Fig. 1. Evaluation of deformability by the diffraction picture resultant from laser beam diffraction in erythrocyte suspension.

In intact rats the erythrocyte deformability coefficient at the maximum shear rate $\gamma \geq 775$ sec^{-1} was virtually the same (0.230±0.002 and 0.230±0.003 in control and experimental groups, respectively, $p>0.05$; Fig. 2, *a*). The erythrocyte deformability coefficient at $\gamma > 775$ sec^{-1} decreased 90 min after surgery by 11±2% in comparison with the initial level and by 12% in rats with cerebral ischemia in comparison with the controls ($p<0.01$), while in sham-operated animals this parameter remained practically unchanged ($p>0.05$; Fig. 2, *b*). Four days after surgery the erythrocyte deformability coefficient at $\gamma \geq 775$ sec^{-1} remained below the initial level (by 16±2%, $p<0.05$) and below the control (by 16%, $p<0.01$; Fig. 2, *c*).

Erythrocyte deformability was impaired in hypoxia of different origin. Decreased capacity to deformation was observed in experimental animals with hind limb ischemia [10] and in humans with ischemic stroke [1]. In rats with experimental cerebral ischemia this parameter remained unchanged for 1 h, but blood viscosity and hematocrit decreased [11]. Changes in erythrocyte morphology and deformability are caused by various factors, including pH changes (decreased concentration of ATP, oxidative stress, formation of hemoglobin and spectrin complexes) [3]. According to published data, the most possible causes of the observed erythrocyte rigidity in cerebral ischemia are LPO-induced changes in phospholipid composition, damage to the protein component of erythrocyte membranes [9], and activation of free radical oxidation. Reactive oxygen species can impair lipid and protein components of erythrocyte membranes and oxidize thiol groups in integral membrane proteins with the formation of cross-links between protein molecules and formation of high-molecular-weight protein conglomerations impeding conformation changes in membrane proteins. These changes deteriorate viscoelastic characteristics of the erythrocyte membrane [13]. It was previously shown that the content NO markedly increases in brain tissues during cerebral ischemia [5].

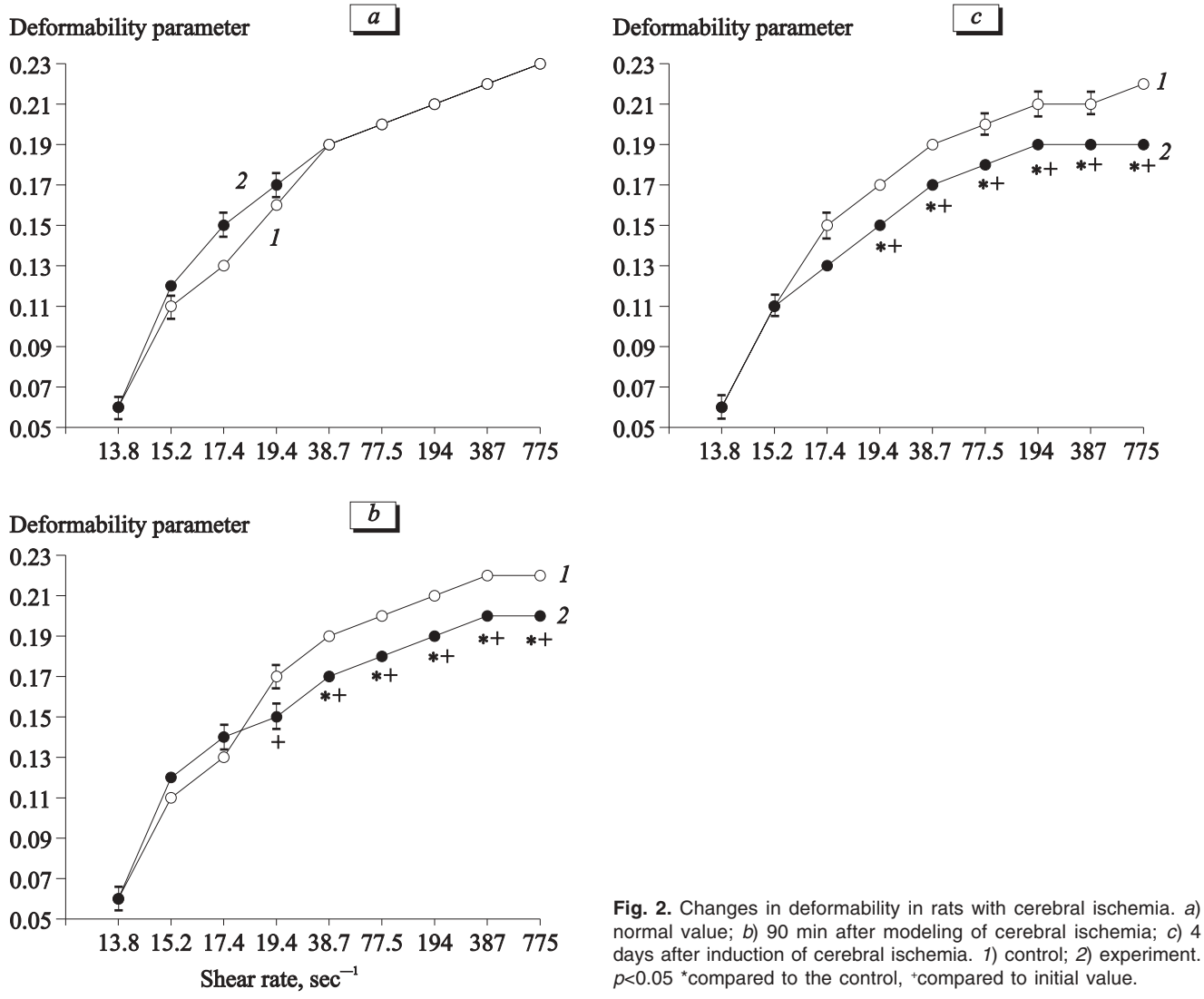

Fig. 2. Changes in deformability in rats with cerebral ischemia. *a*) normal value; *b*) 90 min after modeling of cerebral ischemia; *c*) 4 days after induction of cerebral ischemia. *1*) control; *2*) experiment. $p<0.05$ *compared to the control, +compared to initial value.

These facts are in good agreement with clinical data indicating deterioration of erythrocyte capacity to deformation. Presumably, changes in erythrocyte deformability depend on the severity and duration of ischemia. Decrease of the erythrocyte capacity to deformation in cerebral ischemia can further deteriorate blood supply and oxygen transport in ischemic brain tissue. Rigidity of the erythrocyte membrane and their incapacity to penetrate into narrow capillaries can lead to multiple organ abnormalities caused by deficient oxygen supply to many organs.

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