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MODIFICATION OF THE FUNCTIONAL STATE OF NEUTROPHILIC GRANULOCYTES OF BLOOD DUE TO COAGULATION AND SHEAR STRESS IN PATIENTS WITH CORONARY HEART DISEASE: GENERAL CYTOCHEMICAL PHENOMENA

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A comparative study is made of the influence of a viscosimetric trauma and a coagulation process on the functional state of neutrophilic granulocytes of blood (NGB) in 25 patients with coronary heart disease (CHD). It is shown that different mechanisms of cell activation exist in CHD patients with a stable and unstable course of the disease caused by the viscosimetric trauma effect, which have common features with the NGB activation due to coagulation.

Epidemiological studies carried out in recent decades have demonstrated that some procoagulants (in particular, fibrinogen) represented risk factors for atherosclerosis and its complications [1, 2], independent of the classical risk factors. At the same time the works devoted to the rheological properties of blood put emphasis on increasing the fibrinogen concentration as one of the main factors in increasing the viscosity of plasma and/or of whole blood [2-4]. Moreover, investigation of the classical atherosclerosis risk factors has lead to understanding of the fact that the common mechanism of their adverse influence on the ischemia course is an increase in the plasma viscosity and deterioration of the rheological properties of erythrocytes [5]. The blood flow separation at the sites of convolutions and bifurcations of a vascular bed causes additional damage to a vessel wall due to both the turbulent flows and the increased traumatosis of blood corpuscles, of which the platelets and white blood cells contribute much to the enhancement of endothelium injury and initiation of thrombosis [1, 2, 4-6].

Some experimental studies have revealed the formation of regions with a high shear stress and turbulent flows at the sites of atherosclerotic stenosis accompanied by the formation of a "pale" leucocytic thrombolus, while the blood separation after stenosis favors the development of regions with a low shear stress where a "red" fibrinous erythrocytic thrombus is formed and the phenomen of "leucocyte plugging" is developed in the vessels of a microcirculatory channel. This closes a vicious pathogenetic cycle and aggravates still further the perfusion disturbance [3-5, 7].

The role of the cellular mechanism of the hemostasis system, especially neutrophilic granulocytes of blood (NGB), in the pathogenesis of occlusive-thrombotic CHD complications is still most unclear since the influence of the coagulation processes, like the hemorheological conditions, on the functional state of NGB remains the least understood aspect.

Investigations of the functional response of NGB on the coagulation and fibrinolysis processes [8] have allowed two types of the functional response of NGB to be classified.

The coagulation process in the patients with stable CHD gives a powerful impetus to activation, thus increasing the sensitivity of NGB to activation stimuli, which is accompanied by the development of an adequate secretory response, the degree of which is closely related with the fibrinolytic potential of blood.

On the contrary, in the case of the unstable CHD course the secretory response is depleted due to the appearance in the circulating blood flow of active NGBs capable of spontaneous secretion of the secretum of lysosomic granuli and capture of additional activation signals, among which cytokines, paracoagulation derivative

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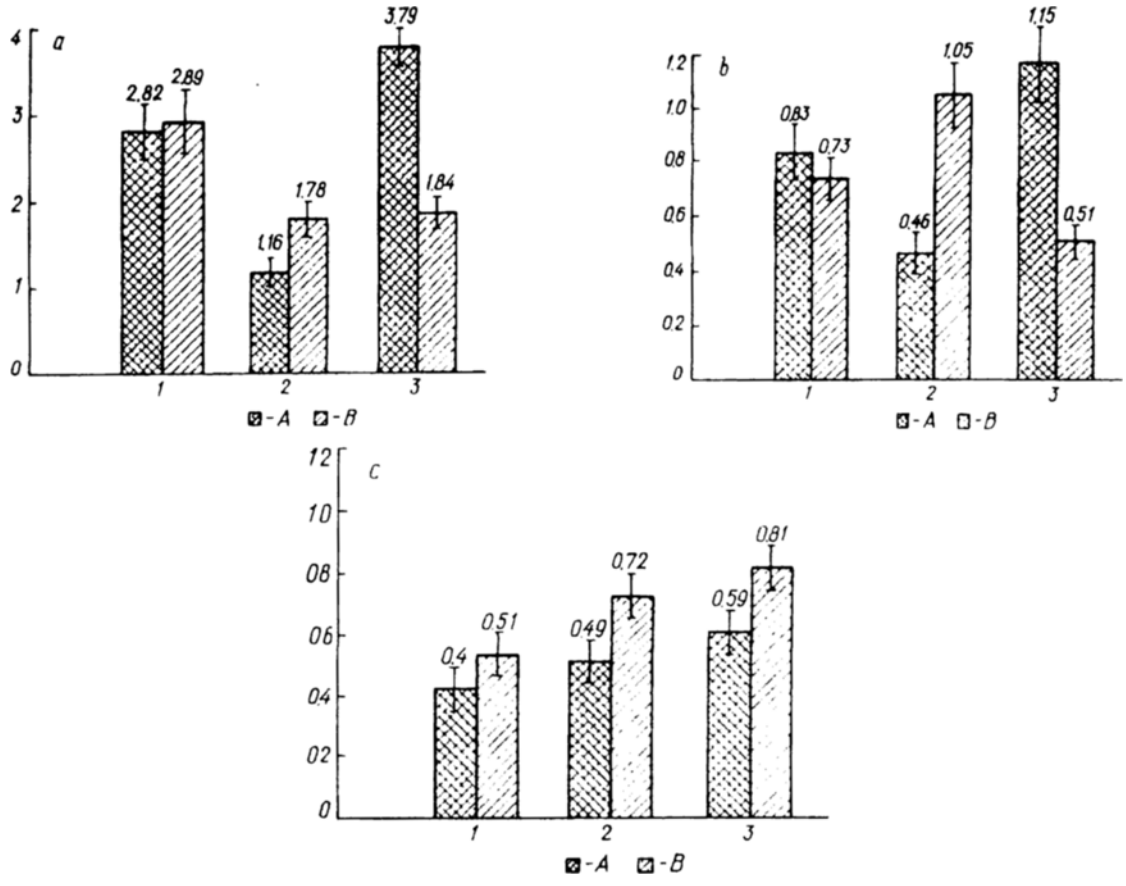

Fig. 1. Intensities of the green (a) and red (b) fluorescence of polymorphonuclear white blood cells as well as their ratio (c) versus the coagulation process and rheological stress: 1) initial value; 2) response to the coagulation process; 3) response to the rheological stress; A, B–1 and 2nd types of the functional response of white blood cells (along the vertical line – relative optical units).

of fibrinogen, and thrombin play an important part [8, 9]. The establishment of the dependence of the types of a functional response of NGB on fibrinolysis in the case of the stable CHD and on the fibrinogen concentration and thrombocyte activity in the hemostasis system in the case of unstable CHD has made it possible to presume the expression of different families of cellular adhesive molecules in different CHD courses, i.e., proteolytic manifestation of leuctin-like sites (*L*-selectins) for the stable CHD, which increases the NGB selectivity to activation stimuli and, as a result, increases the thrombosis risk. In the case of the unstable CHD, the NGB receptors show interest in fibrinogen (cellular adhesive molecules of the integrin family) as a result of the pronounced NGB activation in the circulating blood flow [8, 9].

From the aforesaid it seems urgent to carry out a comparative study of the functional response of NGB to coagulation and viscosimetric trauma under standard conditions set on a rotatory viscosimeter for CHD patients with different climical pictures.

25 CHD patients aged from 45 to 65 (of those 65% are men from 45 to 55) were examined. 10 cases were diagnosed as progressive stenocardia, and 15 as stable stenocardia of tension of the second functional class.

Viscosimetric studies of the samples of human plasma and whole blood with the standardized packed cell volume (0.4 liter/liter) were made on a AKR-2 rotatory viscosimeter (Komed, Moscow) in the shear rate range of 200–20 sec^{-1} at 37°C. In addition to the human blood viscosity investigation, we calculated the erythrocyte deformability (the ratio of the blood viscosity at the shear rate of 200 sec^{-1} to that at 100 sec^{-1}) and aggregation indices (the ratio of the blood viscosity at 20 sec^{-1} to that at 100 sec^{-1}). The shear trauma of the human blood

samples was preset by a rotatory viscosimeter under standard conditions: the shear rate of 200 sec^{-1}, 30 sec exposure, temperature of 37°C.

The functional state of NGB was investigated with the human blood samples performing a luminescent microspectral analysis on a double-wave microfluorimeter-photometer [10], model DMF-2, with supravital staining of blood preparations by 1×10^{-5} M acrydine orange in a phosphate buffer (pH 4.2).

To evaluate the functional modification of cells, the initial state of NGB and changes in microspectral characteristics just immediately after a shear trauma were investigated. The evaluated parameters included the green luminescence intensity (GLI) that reflects fluorochrome incorporation into desoxyribonucleoprotein and characterizes the functional state of the nuclear mechanism of NGB, and the red fluorescence intensity (RFI) that at a given gradient pH characterizes the dye accumulation in a lisosome apparatus. The GFI-to-RFI ratio is an integral parameter of the functional activity of a cell that characterizes acrydine orange redistribution between the mentioned cellular structures [11].

For the purpose of investigating the possible role of membrane damage in the development of viscosimetric NGB trauma concurrently before and after it the spontaneous NGB damage test was conducted by staining the blood preparations with ethydium bromide and counterstaining with acrydin orange, followed by calculation of the percentage of nonviable cells stained by ethydium bromide into the typical red [10, 11].

The initial condition of rheological parameters of the blood samples of the tested patients was characterized by a slight increase in the plasma viscosity at low shear rates, which at 20 sec^{-1} exceeded the reference values (3.3 + 0.11 cP) obtained in viscosity investigations of 10 healthy donors and made 6.30 ± 1.32 cP ($p < 0.05$). The erythrocyte aggregation and deformability indices (2.43 ± 0.82 and 0.98 ± 0.11, respectively), as compared to the reference values (1.10 ± 0.09 and 160 ± 0.23, $p < 0.05$) testified to the pronounced aggregation of red blood cells of the same kind with increasing "rigidity" of their membranes.

However, the results of investigations of the shearing trauma effect on the functional activity of NGB are much more interesting. The microspectral parameters showed heterogeneous changes that are a mirror reflection of two types of the functional response of NGB to coagulation processes. Figure 1 shows the results that allow a comparison of the functional response of 25 CHD patients to shearing trauma and of 77 patients to coagulation processes. The comparison is based on stable CHD cases, as the second type of the functional response to coagulation processes is discovered only in the unstable cases.

The stable CHD-patients belonging to the first type of the functional response of NGB to coagulation processes showed a decrease in the intensities of green and red luminescence in microscope-observed phagosomata with unstained fibrin, which can be considered an adequate secretory NGB response as a result of their preceeding activation during coagulation. In the case of the stable CHD, the response to a viscosimetric trauma is accompanied by the distinct cellular activation, i.e., the statistically appreciable enhancement of the functional activity both of the nuclear (Fig. 1a, A) and lysosomal (Fig. 1b, A) apparatus of NGB, which is visually accompanied by the occurrence of "flattened" as a result of adhesion cells. Such a microfluorimetric picture is characterized by a moderate increase in the integral cellular activity (Fig. 1c, A).

Investigation of the initial NGB viability using the data of the spontaneous damage test of this group of patients did not reveal an increase in the number of affected cells in the samples. It is pertinent to note that the NGB activation in response to a shearing trauma was not accompanied by an increase in the amount of affected cells that did not exceed (as in the initial state) 2%. Thus, the activation due to the shearing trauma was not related with the deep destruction of membranes and a decrease in the viability of cells.

On the contrary, in the patients with progressive stenocardia and the second type of the functional NGB response to coagulation processes (active NGB with spontaneous secretion in a circulating blood flow with depletion of the secretory response to blood coagulation) the microfluorometric and microscopic picture is quite different: there are observed a statistically appreciable depletion of the functional reserve of nuclei (Fig. 1a, B), rapid decay of red luminescence as a consequence of depletion of the lysosomal system (Fig. 1b, B), and much more pronounced "flattening" of cells as a result of the hyperadhesive NGB state at a moderately increased RLI/GLI ratio (Fig. 1c, B). A radically different picture was observed in investigations of the spontaneous NGB destruction: while in the initial state only 1–2% cells were nonviable, after a viscosimetric trauma up to 12% affected cells were found in

the preparations. Thus, in the patients with progressive stenocardia a viscosimetric trauma reduces the NGB vialability even more: the emergence of active cells with spontaneous secretion causes the depletion of functional reserves of this cellular unit, while an additional viscosimetric trauma makes its functional insufficiency even worse, which causes the death of cells and further disorders in the cell-to-cell communication.

Proceeding from the above investigation, the following conclusions can be drawn:

1. Different mechanisms of the cellular activation underlie the NGB participation in the pathogenesis of stable and unstable CHD course.

2. In the case of the stable CHD course, the NGB activation, caused by a shearing trauma upon passage through the zones of a stenotic lesion of coronary arteries, can favor transformation of the stable into the unstable course of the disease: an increase in the NGB sensitivity to activation stimuli is realized through cooperative activation of the vascular-thrombocytic mechanism of the hemostasis system and additional alteration of endothelium, which favors initiation of the thrombotic process.

3. With the unstable CHD course, the appearance of active NGB in a circulating blood flow is not related with a viscosimetric trauma any more since the promoting local processes of thrombogenesis and fibrinogen-mediated NGB activation with spontaneous secretion of lysosomal granules become very important, which probably disturbs the hemostasiological equilibrium and damages the endothelium of a vessel wall even to a greater degree.

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