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# STRUCTURAL AND FUNCTIONAL PROPERTIES OF PLATELETS IN ISCHEMIC HEART DISEASE

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UDC 616.12-005.4-07:616.155.25-07

KEY WORDS: platelet membranes; viscosity of membranes; aggregation; cholesterol; ischemic heart disease.

One stage in the pathogenesis of atherosclerosis is an increase in coagulability of the blood as a result of a disturbance of equilibrium between the clotting and anticlotting systems of hemostasis, in which platelets are the principal key component. Atherosclerosis is accompanied by increased platelet activity, manifested as a decrease in the platelet circulation time, increased sensitivity to aggregation inducers, and a decrease in the thrombus formation time [6, 7, 11, 12]. However, investigations of this kind have been conducted on platelet-rich plasma, and it is not known whether hyperreactivity was the result of certain changes in the properties of the cells themselves or of the humoral action of clotting factors. Another debatable problem is whether the cholesterol (Ch) fraction in the platelet membranes of patients with ischemic heart disease (IHD) and, in particular, in hyperlipidemia, is increased. Whereas investigations (experimental and clinical) have revealed an increase in the molar ratio cholesterol/phospholipids (Ch/PL) in the erythrocyte membranes in atherosclerosis, inducing an increase in microviscosity and inhibiting membrane $Na^+$, $K^+$-ATPase [1], the few data relating to platelets are highly contradictory [10-12].

This paper describes the first attempt to study microviscosity of platelet membranes and aggregation of isolated platelets of patients with IHD and to compare them with the results of determination of the Ch fraction in them.

## EXPERIMENTAL METHOD

Experiments were carried out on platelets isolated from blood of patients with IHD aged from 30 to 60 years. The diagnosis was based on clinical manifestations of angina, ECG changes, and the results of graded physical exercise tests. Blood from healthy blood donors of the same age was used as the control. Blood was taken from the cubital vein into a siliconized tube with 3.8% sodium citrate in the ratio 9:1 by volume. Platelets were isolated from platelet-rich plasma by gel-filtration on sepharose 2B. The state of platelet function was judged by the ADP-induced aggregation time. The rate of platelet aggregation was measured by the method [4], by recording the decrease in scattering of light by a cell suspension in buffer (134 mM NaCl, 15 mM Tris-HCl, 1 mM EDTA, 5 mM glucose; pH 7.35) after addition of $10^{-4}$ M ADP and $3 \cdot 10^{-3}$ M $CaCl_2$ per $10^7$ cells in 1 ml; the time of reaching maximal translucency was estimated at a wavelength of 620 nm and at 37°C. The Ch content in the plate-

Department of Biophysics, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 1, pp. 17-19, January, 1984. Original article submitted April 7, 1983.

 0007-4888/84/9701-0018$08.50 

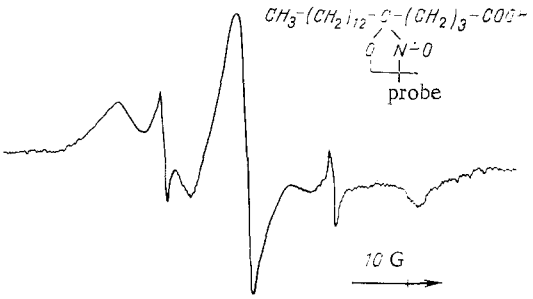


Fig. 1. Appearance of EPR spectrum of spin probe 5-doxylstearate in human platelets.

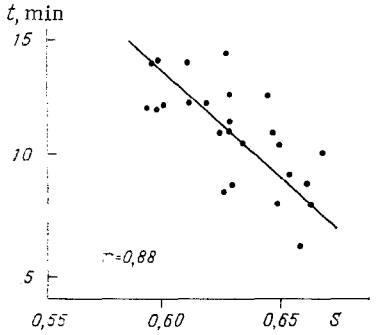


Fig. 2. Dependence of platelet aggregation time (t) on parameter of orderliness (S) of spin probe introduced into platelet membrane.

lets was determined after extraction of lipids by the method [5], and PL by Vaskovsky's test [13]. From the results the ratio Ch/PL was calculated as the molar ratio of Ch to PL in the test samples. Membrane structure was studied by the EPR spin probe method, using a derivative of stearic acid with a nitroxyl fragment in position 5 relative to the carboxyl group. The probe was introduced into the samples in the form of ethanol solutions; its final concentration was $10^{-5}$ M/6·$10^8$ cells/ml, and the ethanol content did not exceed 2% by volume. The EPR spectra were recorded on an E-4 radiospectrometer (Varian, USA) in a thermostatically controlled flat cuvette at 24°C, with microwave power of 10 mW, high-frequency modulation amplitude 1-2 G, and rate of development of the magnetic field 100 G in 8-16 min. To assess the degree of randomness of movement of the acyl chains of PL in the platelet membranes, the parameter of orderliness S [2] was used.

## EXPERIMENTAL RESULTS

A typical EPR spin probe spectrum in human platelets is shown in Fig. 1. The parameter of orderliness S, which depends on the microenvironment and reflects the degree of orientation and mobility of the fatty acid chains of PL at the site of the probe, in the patients with IHD was higher (0.642 ± 0.010) than in healthy human platelets (0.619 ± 0.012; Table 1). As was shown in [9], the fatty acid spin probe under experimental conditions is located entirely in the outer membrane of the platelets; changes discovered in the parameter of orderliness, moreover, indicate modification of the structure of the phospholipid bilayer of the plasmalemma. PL of the cytoplasmic membranes of platelets from patients with IHD thus have denser molecular packing than normally. One cause of the changes observed may be accumulation of Ch. To study this problem the molar ratio Ch/PL was determined in the platelets. It will be clear from Table 1 that in normal subjects it was 0.62, in agreement with the value obtained in other investigations [10, 11], and a little higher than the value given in [12]. In patients with IHD, with a high plasma Ch level, the molar ratio Ch/PL was higher (0.76). The increase in microviscosity of the platelet membranes is thus a result of the condensing effect of Ch.

To discover the effect of modification of the physicochemical properties of platelet membranes on their function, aggregation of the cells induced by ADP in the presence of $Ca^{++}$

TABLE 1. Physicochemical and Functional Properties of Platelets from Patients with IHD and Healthy Blood Donors

| Group tested | Parameter of orderliness S | Ratio Ch/PL | Aggregation time, min |
|---|---|---|---|
| Healthy (n = 20) | 0,619±0,012 | 0,62±0,09 | 12,3±1,1 |
| Patients with IHD (n = 30) | 0,642±0,010 | 0,76±0,08 | 9,4±1,1 |
| P | <0,01 | <0,01 | <0,05 |

ions was studied. The time taken to reach maximal translucency after addition of the inducer was found to be substantially less, and consequently, the rate of aggregation higher, for platelets from patients with IHD than normal subjects (Table 1). The increase in aggregating power of the platelets may be due to increased microviscosity of their membranes. To test this hypothesis dependence of platelet aggregation time on the parameter of orderliness of the spin probe was studied (Fig. 2). It will be clear from Fig. 2 that with an increase in the parameter S and, consequently, with a decrease in flowability of the membranes, the platelet aggregation time decreased.

It might be supposed that one of the main causes of the increased aggregating power of the platelets of patients with IHD is a change in the physicochemical state of their membranes. Besides an increase in the Ch content, there may perhaps also be other mechanisms by which the membrane structure of the platelets affects their aggregation properties [8]. For instance, it was shown in [12] that platelets from patients with hereditary abetalipoproteinemia, which have the highest Ch content (Ch/PL = 1.47), nevertheless do not possess increased powers of aggregation, whereas in Tangier disease platelets with a normal Ch content (Ch/PL = 0.64) aggregate much less strongly than normally. The authors cited consider that it is not Ch which affects aggregation, but the structure of the phospholipid microregions located near the binding site of aggregation stimulators. Other workers [11] also consider that changes in the membrane may affect the position and mobility of membrane components responsible for reception and transmission of aggregation stimulators. These workers, unlike those mentioned above, regard incorporation of Ch as the cause of membrane modification. Meanwhile the experiments of Margolis et al. [3] revealed nonadhesiveness of platelets with lipid films, and which, according to ERP spin probe data, are in a liquid-crystalline state, and maximal adhesion was found with solid (jelly-like) lipids. The data mentioned above, together with the results of the present investigation, suggest that platelets of patients with IHD, which have a more rigid membrane, adhere more readily to other, especially endothelial, cells and this may lead to the formation of juxtamural thrombi, which are associated with atherosclerotic diseases.

The results of the present investigation thus indicate that the increased aggregating power of platelets of patients with IHD is linked with an increase in the packing density of the membrane PL, evidently as the result of a combination of several factors, including the influence of cholesterol.

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