MEMBRANE FLUIDITY OF BLOOD PLATELETS IN MYELOID NEOPLASMS

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Abstract. A large variety of platelets dysfunctions have been described in myeloid neoplasm. In this study we evaluate the alterations in membrane fluidity of blood platelets in patients with various entities of myeloid malignancies. Membrane fluidity was assessed by fluorescence anisotropy measurements. Platelet membrane from acute myeloid leukemia (AML) and myelodysplastic syndrome patients was found to be significantly more rigid compared with that of controls/normal, but it became more fluid if the disease progressed towards a more severe status. We consider that detection of these modifications may be useful for a better insight into cell abnormalities occurring in this pathology.

Key words: membrane fluidity, fluorescence anisotropy, blood platelets, myeloid neoplasm.

INTRODUCTION

The clinical history of myeloproliferative and myelodysplastic disorders is complicated by thromboembolic or hemorrhagic events [8, 12]. Since there is a weak correlation between the risk of these life threatening complications and the number of blood platelets, many researchers are focusing on qualitative defects of platelets. Still, the mechanism of these major complications remains unclear. In research laboratories platelet function is investigated by ultrastructural studies [3], aggregation assay [16, 20, 21, 24], membrane lipid profiling [14] or evaluation of the platelets oxidative response [2, 10, 19, 24]. Membrane fluidity is another important parameter which influences many of the unique cellular functions [1, 4–6, 18, 22] and which is strongly correlated (among other factors) to the membrane lipid composition [7, 15]. In our study we evaluated the alterations in platelet membrane fluidity associated with different myeloproliferative and myelodysplastic disorders, using steady state fluorescence anisotropy meaurements. The myeloid

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neoplasms included in the study were: acute myeloid leukemia (AML group), myelodysplastic syndrome (MDS group) and chronic myeloproliferative disorder (MPD group).

MATERIALS AND METHODS

PATIENTS

We selected 32 patients with various entities of myeloid neoplasm. These patients were diagnosed according to the WHO criteria (Department of Hematology, Emergency University Hospital of Bucharest) [23]. They all gave an informed consent and the study was in accordance with Helsinki Declaration revised in 1983. 11 normal healthy volunteers, non smokers, drug-free, were used as controls.

PLATELET PREPARATION

Platelets were isolated from 5 mL peripheral venous blood obtained by venipuncture on anticoagulant citrate. Sodium citrate was used in order to minimize deterioration of platelet function during experiments [9]. The entire procedure of isolating platelets was carried out in plastic tubes at room temperature. Platelet-rich-plasma was obtained by centrifugation at 700 g, for 5 min. Apyrase (Sigma) was added (final concentration 40 μg/mL), according to [11]; platelets were then washed in Hank's balanced salt solution (HBSS): 145 mM NaCl, 5 mM KCl, 10 mM Hepes, 1 mM MgSO4, 10 mM D-glucose, pH 7.4. A final centrifugation at 1500 g for 10 min was carried out and the pellet was resuspended in HBSS. Until the measurements were performed, samples were kept at 37 °C, for approximately 20 min on a thermostated circulator stirrer (Unimax 1010, Heidolph) (60 rpm). Platelets were used within 2–4 h after blood collection.

There were patients and volunteers who gave two blood samples at different time points during the study.

FLUORESCENCE ANISOTROPY MEASUREMENTS

The membrane fluidity was estimated by steady state fluorescence anisotropy measurements, using the amphiphilic fluorescent probe N,N,N-Trimethyl-4-(6-phenyl-1,3,5-hexatrien-1-yl)phenylammonium p-toluenesulfonate (TMA-DPH) (Sigma). The cells were incubated in 0.1 mM TMA-DPH (final concentration) for 5 min before each experiment and fluorescence intensities (λ_{ex} = 355 nm, λ_{em} = 425 nm) were measured on a computer-connected spectrofluorometer (Jobin-Yvon) under continuous stirring at 120 rpm. For each sample, 3 measurements at room

temperature were performed. The background fluorescence (TMA-DPH in HBSS in the absence of cells) was found to be negligible.

Steady-state fluorescence anisotropy (r) was calculated using the formula:

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \quad (1)$$

where I_{VV} and I_{VH} are the measured vertical and horizontal components of the emitted light respectively, when the sample is excited with vertically polarized light [11], while G is a correction factor which is calculated as the ratio I_{HV}/I_{HH} (same meaning but using horizontally polarized excitation light). Fluorescence anisotropy is correlated with the wobbling movement of the probe within the membrane; a low r value indicates a high mobility of the microenvironment around the probe (i.e. high membrane fluidity).

Statistical analysis. The statistical significance of differences between groups was analyzed by Student's t-test, a value of $p < 0.05$ was considered significant. Results are expressed as means ± standard deviation (SD).

RESULTS

From the 32 patients, 15 were diagnosed with AML, 8 patients with MPD and 9 patients with MDS. We totalized a number of 56 samples from these patients and 18 samples from volunteers.

Changes in membrane fluidity were significantly different in AML and MDS group, compared to controls (Table 1). For AML and MDS patients the platelets membranes were more rigid than for controls. While between MPD and control groups and between the pathological groups the differences in membrane fluidity were not statistically significant.

Table 1

Fluorescence anisotropy values of platelets for control and different pathologies groups. Mean value of *r* was calculated using all 56 samples from patients and 18 samples from volunteers, respectively. For patients with AML and MDS significant differences could be seen when compared to controls ($p < 0.05$)

Pathology	**Control**	**AML**	**MDS**	**MPD**
mean $r \pm SD$	0.160 ± 20.026	0.201 ± 0.053	0.192 ± 0.039	0.185 ± 0.048
p	–	0.007	0.014	0.102

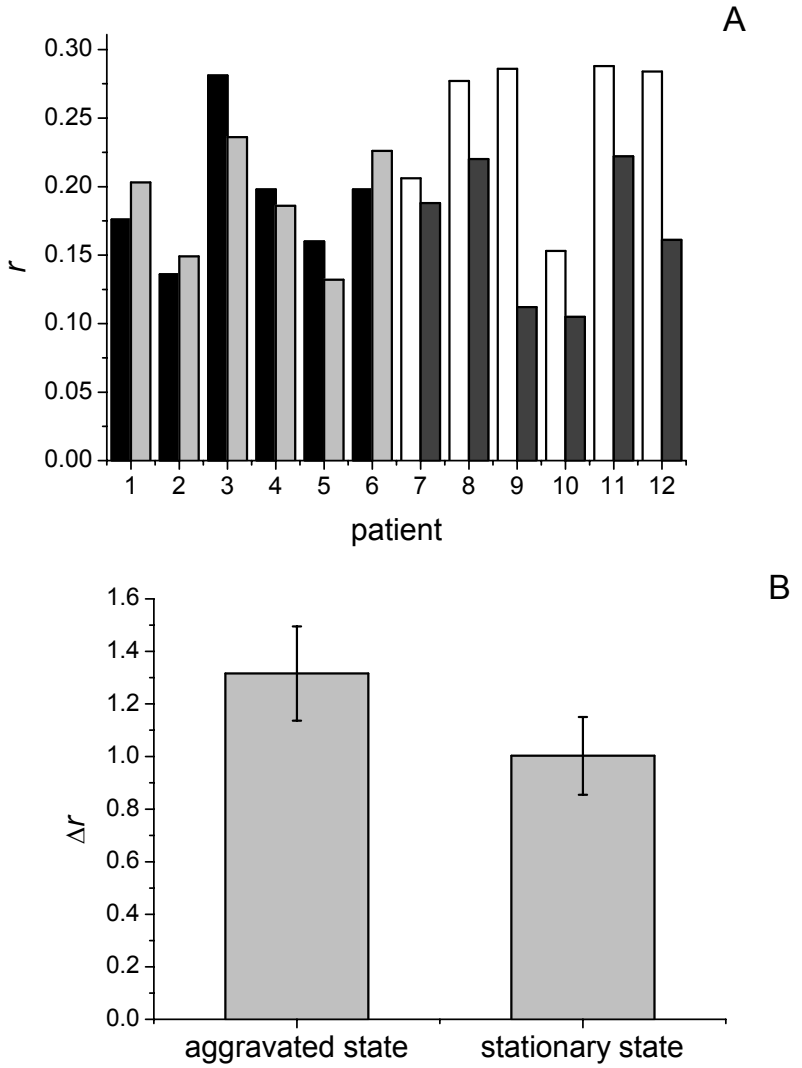

Fig. 1. Comparison of fluorescence anisotropy (r values) for platelets membrane from 12 patients with acute myeloid leukemia. Fluorescence anisotropy was measured at two different moments during the period of study, over a period of one to six months. A) patients 1 to 6 are characterized by stationary disease (initial anisotropy in black bars and final anisotropy in light grey bars) and patients 7 to 12 with aggravated clinical state (initial anisotropy in white bars and final anisotropy in dark grey bars; B) r value decreases (Δr increses) for patients in aggravated state of AML disease ($p = 0.058$ when compared to stationary state).

We compared the fluorescence anisotropy during the disease evolution for 2 groups of patients: one group consisted of 6 patients with stationary AML (*de novo* AML or secondary due to MPDs) and another group of 6 patients who developed a

more severe type of AML (clinically assessed). 3 patients from AML group died during the assessment period. Results are shown in Figure 1A, where fluorescence anisotropy evolution over 1 to 6 months for the first group of patients is represented in black and white bars, while that for the second group in dark and light grey bars. One can observe that r value constantly decreased (with 10 to 53%) for patients whose disease state aggravated during the above mentioned period. In order to quantitatively compare the two groups of patients we propose the following formula:

$$\Delta r = 1 + \frac{r' - r''}{r'} \tag{2}$$

where r' is an initial measurement while r'' is measured after a period of 1 to 6 months (for the same patient). According to this formula, if the r value for a patient decreases during the disease evolution (membrane becomes more fluid), the Δr value will be higher than 1, and vice versa. The mean $\Delta r \pm SD$ values for AML patients in stationary and aggravated disease states are presented in Figure 1B. The difference in Δr values between AML patients in these two disease states was close to the statistical significance threshold ($p = 0.058$), one may consider that the platelet membrane of AML patients in aggravated state is less rigid than in stationary AML patients.

DISCUSSION

According to our results the platelets membranes from patients show to be more rigid regardless of the clinical type of myeloproliferative disorder. Patients with severe clinical status due to AML have a less rigid membrane compared to the same patients previously in a better state. Thus, the activity (or severity) of the disease correlates with the increase in membrane fluidity, as other studies revealed on lymphocytes [1].

Biological membranes are complex assemblies of lipids and proteins, with functionally specialized domains; changes in membrane parameters (including fluidity) may thus perturb functioning of receptors and ion-channels. TMA-DPH, a fluorescent marker intensively used for membrane fluidity studies, is membrane incorporated at the lipid-water interface [13]. A decrease of platelets membrane fluidity, as that observed in our patients, reflects an increase in lipid order in the external leaflet of the platelet membrane.

Previous studies of platelet membrane fluidity have shown that platelets activation is associated with changes in the mobility of proteins and lipids within the membrane [18, 22]. So, differences in microviscosity observed in our study may be caused by platelets activation. By now, only a few studies are focused on the modifications of platelets membrane fluidity in patients with hematological

malignancies. For example, a study by Leoncini *et al.* found an altered pattern of fatty acids in platelet membrane of patients with thrombocytosis due to myeloproliferative disorders which may decrease the platelet membrane fluidity [14].

The experiments in this study were made *ex vivo*; within the organism, the changes in membrane fluidity are much more complex and during cell preparation many of membrane properties, including fluidity, may change. Also, the study of platelets in myeloid neoplasm is complicated by: (i) the difficulty in obtaining sufficient amount of platelets for measurements, (ii) the associated pathology and (iii) the medication. Little is known about membrane adaptation to drugs, so it is uncertain whether the course of treatment was sufficiently long to alter the data.

CONCLUSIONS

Platelet membrane fluidity was studied in several other pathologies (e.g. diabetes mellitus, ischemic heart disease, acute stroke), but less in the case of myeloid neoplasms, even though it is known that membrane fluidity may influence many cellular functions, being correlated to the membrane lipid composition [17].

Membrane fluidity modifications were detected also in other blood cells from leukemic patients [1, 4–7, 15].

Our experiments showed that platelet membrane from acute myeloid leukemia and myelodysplastic syndrome patients was significantly more rigid compared with that of controls. The membrane fluidity changed depending on the evolution of the disease becoming more fluid if the clinical status of the patient became more severe.

We consider that their detection in platelets is useful for a better understanding of cell abnormalities occurring in these pathological conditions.

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