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Impairment of the erythrocyte membrane fluidity in survivors of acute myocardial infarction. A prospective study

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Abstract. Erythrocytes have to constantly adapt themselves to the varying circulatory system shear stress forces and capillaries diameter. Membrane lipid and protein content have an important role in determining the erythrocyte shape and are main determinants of the membrane solid and fluid behaviour which enables the erythrocyte to respond to the outer environment modifications. Membrane fluidity is an inverse index of membrane microviscosity. The aim of the present work is to evaluate prospectively in three periods of time (discharge, after 6 months and one year later) in survivors of an acute myocardial infarction (AMI) the erythrocyte membrane fluidity (outer and inner bilayer) and establish a relation with the cardiovascular events or need of coronary revascularization during a two year clinical follow up.

Sixty survivors of acute myocardial infarction were recruited during 1994–96 and were prospectively studied in three periods (discharge, 6 months and after one year), and were compared with a control group ($n = 36$). Membrane lipid fluidity was determined by means of fluorescence polarisation with two probes: 1,6-diphenyl-1,2,5-hexatriene (DPH) and 1,4-trimethylamine 6-phenyl hexa-1,3,5-triene (TMA-DPH), for the characterisation of the hydrophobic and external polar region, respectively.

The hydrophobic region was more rigidified ($p < 0.01$) in the erythrocytes from AMI patients, in relation to the control group. During the time of the study there was a progressive erythrocyte membrane rigidification (DPH $p < 0.001$; TMA-DPH $p < 0.001$). We found no relation between erythrocyte membrane fluidity and the coronary risk factors, cardiovascular events or the need of coronary revascularization during the clinical follow-up.

In conclusion, after the myocardial infarction erythrocyte membrane of AMI survivors becomes more rigid with time, which could contribute to the decreased erythrocyte deformability and the increased blood viscosity previously described in this group of patients.

Keywords: Erythrocyte membrane fluidity, acute myocardial infarction, prospective study

1. Introduction

Erythrocytes have a discoid shape that enables them to deform passively when crossing narrow capillaries and adapt to the continuous variation of the circulatory system shear stress forces. The capacity to reversibly change their shape is crucial for the erythrocyte's oxygen delivery optimisation. Erythrocyte membrane ultrastructure analysis has revealed that cellular membranes are supramolecular noncovalent structures with metabolic activity, dynamic and asymmetric properties. They are composed of two main macromolecules: phospholipid bilayer and membrane proteins.

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The erythrocyte membrane plays a critical role in determining both cell shape and its ability to deform, however transmembrane, cytoskeleton, and cytoplasmatic proteins as well as lipids are major determinants of the functional regulatory process [1,2].

The lipid and protein mobility is another important factor for the dynamic properties of RBC membrane. The association of the solid and fluid behaviour of both protein and lipid was responsible for membrane viscoelastic properties [3], which are prime determinants of the erythrocyte's deformability. Proteins are responsible for membrane elastic properties while viscous properties are influenced by lipid microviscosity [4].

Membrane lipid fluidity is an indirect and inverse index of membrane's microviscosity and lipid mobility, being influenced by several substances such as anesthetics [5], anti-arrhythmic drugs [6], and insulin [7]. In addition, it has been implicated in the modulation of several functions of the erythrocyte membrane, such as (i) increased permeability to sodium with decreasing microviscosity [8], (ii) decreased erythrocyte deformability with increasing microviscosity [9], and (iii) limitation of protein lateral mobility with increased membrane microviscosity [10].

Patients survivors of acute myocardial infarction are at risk for new ischemic events such as angor, reinfarction, stroke; which may be associated with microcirculatory and hemorheological abnormalities, such as increased whole blood viscosity [11], increased plasma viscosity [11], decreased erythrocyte deformability at low shear stress forces [12], and erythrocyte membrane rigidity [13] that have been described at hospital discharge. These abnormalities have been associated with AMI survivors' long-term clinical prognosis [14].

Besides the detected hemorheological abnormalities during the acute phase of an AMI and in patients with coronary artery disease, we still do not know how the profile evolves after the patients' hospital discharge.

The aim of the present work is to prospectively evaluate erythrocyte membrane fluidity variation in three periods of time (discharge, 6 months and after one year) in a group of survivors of an acute myocardial infarction (AMI) proceeding from a coronary unit and study a possible relation with new ischemic events or the need of coronary revascularization. The results are to be compared with a control population of blood donors.

2. Material and methods

Population. Sixty survivors of an AMI (51 men and 9 women) mean age 57.9 $\pm$ 12.2 years old were recruited during 1994–96 from a hospital coronary unit and prospectively studied in three periods of time: hospital discharge ($n = 60$), after 6 months ($n = 47$) and one year later ($n = 30$). The control group was constituted by 36 apparently healthy subjects (24 men and 12 women) mean age of 45.6 $\pm$ 9.8 years old, blood donors of the Hospital Santa Maria blood centre.

Vascular events definition. Two groups of events were collected: (i) vascular events (death, angor, AMI, cardiac failure and stroke) or (ii) need of coronary revascularization (patients submitted to elective coronary aortocoronary bypass and percutaneous coronary angioplasty). These data were processed separately and in association (these patients were considered to be at higher risk).

Methods. Erythrocyte membrane fluidity was evaluated by measuring fluorescence polarisation using two fluorescent probes: 1,6-diphenyl-1,2,5-hexatriene (DPH) and 1,4-trimethylamine 6-phenyl hexa-1,3,5-triene (TMA-DPH) for the characterisation of the hydrophobic and external polar region of the

phospholipid bilayer, respectively [15,16]. A spectrophluorometer Hitachi FL 300 was used. Higher values of the fluorescence polarisation mean lower membrane fluidity, thus higher microviscosity or rigidity.

Statistical analysis. The variation during the time of the study and between groups comparisons were determined with ANOVA variance analysis with the Bonferroni correction. Paired t-test was used for paired comparisons. A 0.05 significant level was considered.

3. Results

3.1. Comparison between erythrocyte membrane fluidity in the control group and AMI survivors (discharge, after 6 months and one year later)

There was no significant variation in the values of the fluorescence polarisation for the external polar region, between the control group and the AMI patients in the three periods of time.

However, the values of the fluorescence polarisation for the DPH probe were higher ($p < 0.01$) in the AMI survivors (for the three periods of time) in relation to the control group. Therefore, the erythrocytes from AMI survivors at discharge, after 6 months and one year later have a more rigidified hydrophobic bilayer than the erythrocytes from apparently healthy subjects.

3.2. Erythrocyte membrane fluidity variation during the three periods of time (discharge, after 6 months and one year later) in AMI survivors

There was a significant variation of the fluorescence polarisation values of the DPH ($p < 0.001$) and TMA-DPH ($p < 0.001$) probes during the time of the study determined with ANOVA analysis of variance. By the Bonferroni correction for multiple group comparison we determined that the previous time-dependent variation was mainly determined by the difference between the discharge values and the two other determinations (at 6 months and after one year). We found no significant difference between the sixth month and the one-year later determination. So the erythrocyte membrane (hydrophobic and external polar bilayers) fluidity increased (rigidified) abruptly after discharge and stabilised after 6 months.

3.3. Erythrocyte membrane fluidity relation with the coronary risk factors in AMI survivors

Fifteen percent of the patients had no coronary risk factors (Table 1). We found no significant relation between erythrocyte membrane fluidity and the previous coronary risk factors or the number of risk factors.

3.4. Erythrocyte membrane fluidity relation with coronary events and need of coronary revascularization

During the time of this study there was a total of 17 cardiovascular events, 19 patients needed coronary revascularization (11 bypass surgery and 8 angioplasty) and one patient died.

There was no statistical difference in the erythrocyte membrane fluidity determined at hospital discharge for the patients who had cardiovascular events and/or need of coronary revascularization. Nevertheless, those who had cardiovascular events during the clinical follow-up had marginally ($p = 0.049$) higher values of the membrane fluorescence polarisation concerning the TMA-DPH at the sixth month determination.

Table 1
Clinical profile of acute myocardial infarction survivors

$N = 60$		
Sex: 51 men; 9 women		
Age: 57.9 ± 12.2 years		
Risk factors	Angor	41.7%
	Previous AMI	16.7%
	Diabetes	13.3%
	Art. hypertension	50.0%
	Smoking	38.3%
Risk factors by patient	0. . .14.7%	2. . .33.7%
	1. . .34.7%	3. . .14.7%
Infarction location	Anterior	40%
	Inferior	41%
Killip class	1 . . . 76.8%	
	2 . . . 15.8%	
Thrombolysis	37.9% (27 with reperfusion criteria)	

Table 2
Values (mean ± SD) of red cell membrane fluorescence polarisation (assessed by two fluorescence probes: DPH and TMA-DPH) in a control group and in survivors of an acute myocardial infarction (AMI) at hospital discharge, after 6 and 12 months later (see text for more details). There was a significant variation of the erythrocyte membrane fluidity in the AMI patients during the time of the study (discharge, after 6 months and one year later)

Probes	Membrane fluorescent polarisation (p)*				Significance level**
	Control group	Discharge	6 months	12 months	
DPH	0.267 ± 0.03	0.298 ± 0.022	0.318 ± 0.022	0.309 ± 0.025	0.001
TMA-DPH	0.325 ± 0.023	0.312 ± 0.027	0.337 ± 0.015	0.333 ± 0.023	0.001

*Fluorescence polarisation value (see material and methods for details). **Variation during the time of the study (discharge, 6 months and one year later).

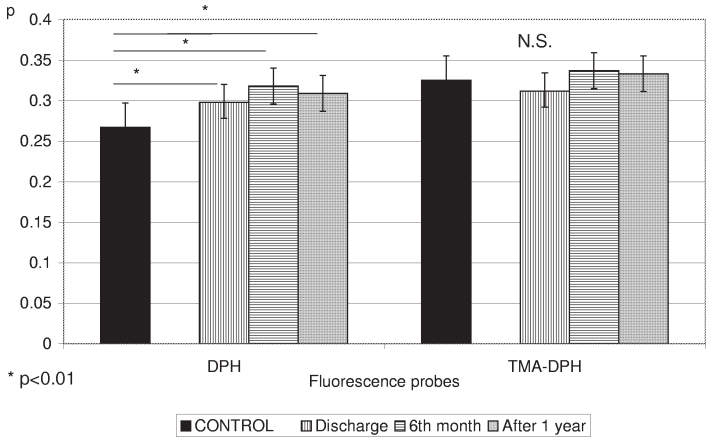

Fig. 1. Erythrocyte membrane fluorescence polarisation (p) in a control group and in acute myocardial infarction survivors determined in three periods of time (hospital discharge, 6 months later and after one year). The fluorescence polarisation values for the DPH probe, which characterises the membrane hydrophobic region, were higher ($p < 0.01$) in the acute myocardial infarction survivors (for the three determinations) than the control group.

4. Discussion

Our group of acute myocardial infarction survivors exhibits at discharge, in relation to the control group, erythrocyte inner membrane rigidification, and no variation of the external polar region fluidity. This asymmetric behaviour of the erythrocyte membrane fluidity could be related with erythrocyte membrane interaction with plasma proteins, such as acute phase reactants. We did not find any relation of the erythrocyte membrane fluidity with the patients therapeutics (in the hospital and after).

After hospital discharge there was a progressive raise of the fluorescence polarisation values in both membrane layers. So, the erythrocyte membrane becomes rigidified. Nevertheless, only the hydrophobic region was more rigid in the erythrocytes from AMI in relation to the control group during the three periods.

This finding could explain the previous reported decreased erythrocyte deformability at low shear stress [12] in AMI survivors at hospital discharge. Erythrocyte deformability at low shear stress is mainly dependent on the erythrocyte membrane rigidity [9,17]. In addition, it could contribute to the erythrocyte aggregation impairment. Nevertheless, these finding could be related to the fibrinogen high concentration seen in these patients. However, it has been reported that fibrinogen concentration lowers after hospital discharge while erythrocyte aggregation increases after hospital discharge [11]

Apparently, no statistical differences were determined for the erythrocyte membrane fluidity in the patients who developed any cardiovascular events or who had previous coronary risk factors. These observations could be related with the small population number or to the small follow-up time. In addition, we found no relation with the patients' cardiovascular risk factors.

In conclusion, this group of acute myocardial infarction survivors, independently of their previous cardiac risk factors and the future development of cardiac events, has rigidified erythrocyte membrane in relation to a reference population. The fluorescence polarisation value for the two probes tends to increase over time, indicating a progressive erythrocyte membrane rigidification process. The present study helps to interpret the evolution of the erythrocyte membrane fluidity in survivors of an acute myocardial infarction, during three periods: discharge, after 6 months and one year later.

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