Original Article

The Role of Atomic Force Microscopy in the Study of the Properties of the Erythrocyte Membrane

Asiyat Hanmagomedovna Neftullaeva[1], Imam Nasirovich Velibegov[1], Khadizhat Nabigullayevna Kurbanova[1*], Madina Akhmedovna Magomedalieva[1], Aminat Alievna Alieva[1], Sardan Zhalilovich Adoi[2]

[1]Department of Therapy, Medical Faculty of Dagestan State Medical University, Makhachkala, Republic of Dagestan, Russia. [2]Department of Therapy, Medical Faculty of Stavropol State Medical University, Stavropol, Russia.

Abstract

A comparative analysis of the molecular organization and functions allows us to determine typical disorders of the erythrocyte membrane in various pathological processes and conditions. One of the promising methods for measuring the mechanical characteristics of erythrocytes and erythrocyte membranes is atomic force spectroscopy. This scientific article describes an experiment in which three donors took part. Using an atomic force microscope, the stiffness of the erythrocyte membrane was evaluated in the case of control, with the addition of hemin, as well as hemin in combination with perfluorane. It was found that the use of hemen increases the average stiffness of the erythrocyte membrane by 2.1 times, and the subsequent use of perfluorane returns the indicator to its original value. Thus, perfluorane partially restored the initial stiffness in 85% of cases. This method can be effectively used to measure the stiffness of membranes under the action of modifiers of a different nature, as well as in the study of erythrocyte membranes in clinical conditions.

Keywords: Erythrocyte, Membrane, Atomic force spectroscopy, Hemin, Perfluorane

Introduction

One of the traditional approaches of pathophysiology aimed at obtaining fundamental knowledge about the general patterns and features of the functioning of cellular systems in pathology is the use of comparative analysis of the molecular organization and function of any cellular structure in diseases of different genesis [1]. This methodology made it possible to determine typical violations of the erythrocyte membrane in various pathological processes and conditions [2, 3]. The choice of the erythrocyte membrane as the object of research was dictated by the fact that it is characterized by the general principles of the molecular organization of plasma membranes [4, 5]. Therefore, the patterns of changes in the structure and function of the erythrocyte membrane with a certain degree of correction, primarily due to the species specificity of cells, can be extrapolated to other membrane systems. In addition, the apparent simplicity of the organization of the erythrocyte as a "degraded" cell makes it possible to study the functional properties of the plasma membrane without interference imposed by intracellular membrane formations and organelles [6, 7]. On the other hand, the erythrocyte membrane has several specific features that determine the functions of these cells, associated with many pathologies and, often, having a purpose and properties unknown to researchers [8, 9].

For a long time, the erythrocyte membrane seemed to researchers to be only a shell permeable to blood gases, separating hemoglobin from plasma [10]. However, the successes achieved in membranology allow us to take a different look at the role of the membrane in the work of the cell. The plasma membrane of the erythrocyte is the most important element of the cell. It is simultaneously a mechanical shell with regulated physical properties and a "control room" that coordinates the work of the cell depending on the physical and chemical signals coming to it in the body [11]. The biological membranes of eukaryotic cells, including the plasma membrane of the erythrocyte, have common structural features: they are ensembles of lipid and protein molecules held together by non-covalent interactions [12]. Thanks to these interactions, the structural integrity of the membranes is maintained: However, cell membranes themselves are mobile, "fluid" structures and

Address for correspondence: Khadizhat Nabigullayevna Kurbanova, Department of Therapy, Medical Faculty of Dagestan State Medical University, Makhachkala, Republic of Dagestan, Russia.
bucky99@ya.ru

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most of their constituent molecules can move in the membrane plane **(Figure 1)** [13, 14]. First of all, it is necessary to consider the structure and organization of the main components of all biological membranes – lipids, proteins, carbohydrates, and their compounds [15].

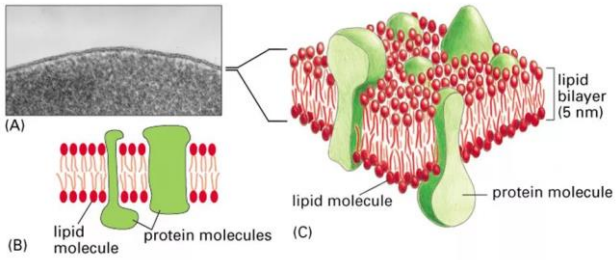

Figure 1. Schematic three-dimensional image of a small area of the erythrocyte membrane

One of the important tasks of the study of blood microrheology is to determine the mechanical properties of erythrocytes. One of the promising methods for measuring the mechanical characteristics of erythrocytes is atomic force spectroscopy. This method is implemented using atomic force microscopes (AFM) [16]. With the help of AFM, force curves are obtained – graphs of the dependence of the elastic force acting on the cantilever from the side of the cell membrane [17]. The study of biological objects on AFM is a technically and methodically complex direction since living cells are soft objects that deform under the influence of force from the cantilever. The measurement of the stiffness of erythrocyte membranes makes it possible to assess the deformability of erythrocytes [18]. The deformability index depends on several cell parameters, in particular on the stiffness of the membrane, the viscosity of the cytoplasm, and the shape (surface area) of the cell [19]. The change in the shape of cells from discocytes to echinocytes and the further development of degenerative forms lead to an increase in blood viscosity and a decrease in the deformability index of erythrocytes [20, 21]. The greater the stiffness of the membrane, the smaller the value of the deformability index. The occurrence of several diseases is accompanied by a change in ID by 15-20% [22]. With injuries and blood loss, ID changes by 10-15% [23], and diabetes mellitus by 20-25% [24]. In turn, the deformability of membranes largely determines the microrheological properties of blood and is the main influencing factor on the viscosity of blood in capillaries [25]. The purpose of this scientific work is to investigate the average local stiffness of the membrane under the influence of membrane modifiers.

Materials and Methods

Blood sampling was performed from three healthy donors during preventive examinations in microvettes containing ethylenediaminetetraacetic acid.

To prepare the working solution, dry hemin was used: 50 mg of dry hemin was dissolved in 1 ml of solution (NaOH), and 5 ml of distilled water was added. The final concentration of hemin in the blood in our experiments was 1.8 mM. The monolayer of cells was obtained using the Swelab Alfa Auto Sampler hematological analyzer (Sweden).

Images of cells and their membranes were obtained using Nano-Observer CS Instruments (Germany) in semi-contact mode.

The stiffness of the membranes was evaluated using the atomic force spectroscopy method. The method makes it possible to measure the amount of deformation of the membrane and cantilever surface depending on the vertical displacement of the piezostole on which the membrane is placed. The average stiffness of the cell membrane was evaluated in the work. Then the values of the average stiffness for n cells were estimated and the stiffness distribution was obtained and the corresponding histograms were constructed.

Results and Discussion

Figure 2 shows an example of obtaining a force curve for measuring the stiffness of the membrane. The angle of inclination of the chord of arc II [nA/nm] was measured, and the stiffness of the erythrocyte membrane section was evaluated. In this series of experiments, erythrocytes were affected by a membrane nanosurface modifier — hemin (hydrochloric acid hematin). Hemin disrupts the conformation of spectrin, the band 4,1 protein, and weakens the bond between them. Hemin was added to the blood in vitro. The concentration of hemin in the blood was 1.8 mM.

The total number of measurements of the local stiffness of the membrane (hemin modifier) is presented in **Table 1**.

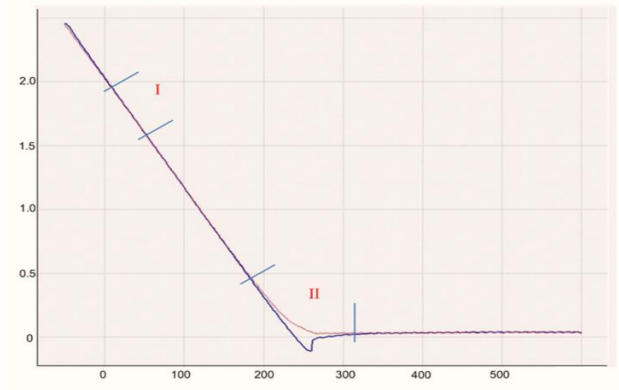

Figure 2. Curves for measuring the stiffness of the membrane: along the abscissa axis — the distance between the cantilever probe and the membrane surface (nm); along the ordinate axis — the value proportional to the interaction force (pA); the red curve — the forward stroke, the blue curve — the reverse.

Table 1. Sample for measuring the local stiffness of the membrane

The investigated parameter	Number of donors	Number of smears for each donor	Number of scans 100x100 microns	The number of test cells in each smear	Number of local measurements per cell	Total number of objects	
						Cells	Points
Control	3	2	2	48	3	576	1728
Hemin	3	2	2	56	3	672	2016
Hemin + perfluorane	3	2	2	58	3	696	2088

The values of the average local stiffness in relative units are given in **Table 2**.

Table 2. Average local stiffness of the membrane after exposure to hemin

The investigated parameter	Control	Hemin (68% of measurements)	Hemin (32% of measurements)	Hemin + perfluorane (85% of measurements)	Hemin + perfluorane (15% of measurements)
K (rel. un.)	4,3±2,6	9,1±2	4±2	4,1±2,6	10±1

The relative stiffness values (K) were calculated as the ratio (tg_{II}/tg_{I}) xM, where tg_{II} and tg_{I} are the tangents of the slope angles of the curve in sections II and I **(Figure 2)**, respectively, the proportionality coefficient M=10.

After exposure to hemin in 68% of cases, the average stiffness increased by 2.1 times compared to the control average, which could reduce ID by almost 30%. In 32% of the cells, the average stiffness of the membrane did not differ from the average control value. Subsequent exposure to perfluorane returned the stiffness of the membrane in 85% of cases to the initial values. The membrane stiffness of the remaining 15% of the areas on the cells remained high — 2.3 times greater than the control, even despite the effect of perfluorane.

Conclusion

Thus, using atomic force spectroscopy, the average local stiffness of the membrane was measured, which depended on the effect of membrane modifiers on it, in particular hemin. This modifier increased stiffness by 2.1 times for most cells. At the same time, the deformability index decreased by almost 30%. Perfluorane partially restored the initial stiffness in 85% of cases. This method can be effectively used to measure the stiffness of membranes under the action of modifiers of a different nature, as well as in the study of erythrocyte membranes in clinical conditions.

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