Abstract

It was proposed a physiologically and experimentally confirmed explanation of Fåhraeus-Lindqvist-effect in capillaries using osmotic profiles of erythrocyte deformability. It was shown the dose-dependent changes of the erythrocytes deformability after forming artificial water pores (nystatin) and occlusion (PbCl2) of available. The effect was conditioned by interchange of the liquid phases between the erythrocyte and plasma in shear flow.

Keywords: Erythrocytes; Deformability; Aqua Pores; Shear Stress

Introduction

The effect of hematocrit changes in small vessels with a diameter less than 150 microns (R.Fåhraeus-effect) and reducing blood viscosity with decreasing size of the vessel (R.Fåhraeus-T.Lindqvist-effect) [1], first described by the authors in 1931, caused attention to theoretical researches and practical modeling. So, erythrocytes in axial cylindrical flow tube slide in the surrounding layer of plasma. This leads to poor marginal zone cells, which accelerates the movement of the liquid core. Thus, the effect is due to the displacement of red blood cells in shear flow and the plasma acts as a lubricant layer [2-4]. These conclusions are consistent with modern hydrodynamics concepts, but the redistribution of particles in the bloodstream does not change the ratio of the solid and liquid phases. The real reason for the change in hematocrit and plasma viscosity the blood flowing in small vessels, remains enigmatic. We aimed to elucidate the physiological mechanism of changes in hematocrit and blood viscosity due to increased shear stress and the associated process of deformation changes of erythrocytes.

Materials and Methods

As is known, the most important determinants of the deformation properties of red blood cells are the internal viscosity of the contents or the degree of hydration of hemoglobin, the ratio of surface area to volume, or S / V, and the degree of rigidity of the membrane. In our opinion, the degree of deformability depends also on the presence of lipid membrane pores through which the liquid phases can be exchanged between the internal and external environments of cells in a changing shear stress.

The studies were carried out by method of gradient ektacytometry[5] in installation of its own production. The shear rate and shear stress in the gap Couette with viscosity 10 cP at 100 rev/min correspond to 1.050 S-1 and 10.5 N/m², which are close to the conditions of the blood flow in the capillaries [6,7]. Osmotic deformability profile or osmokan characterizes changes deformability index Ie versus the osmolality of the suspension medium. Light intensity at high (A) and small (B) axes of the first diffraction ring was measured, and the elasticity (A-B/A+B) or I was calculated. On the chart were identified several characteristic points: Oₘₐₓ-osmolality at which the highest Ie corresponds to isonicity value in blood, Oₘᵢₙ- osmolality at which the minimum Ie observed, it is an accurate measure of the surface-to-volume ratio of erythrocyte population (S/V), and Iₘᵢₙ- deformability in the point of isotropic erythrocyte swelling.

We used 10 laboratory Wistar rats. The blood was collected after decapitation, heparin used as an anticoagulant (100 U/ml). After centrifugation at 600 g for 10 min the plasma was removed, and the erythrocytes were washed once with HEPES-buffered physiological solution (in mM): 145 NaCl, 7.5 KCl, 10 glucose and 10 HEPES at pH 7.4. Water channels were blocked by HgCl₂ (SIGMA-ALDRICH) in concentrations of 2(10⁻⁵-10⁻⁵) on the phosphate buffer. Erythrocytes and buffer with various concentrations of HgCl₂ were mixed in equal proportions and were incubated in 1 hour at 25°C. Water channels in the erythrocytes were formed with help of polyene antibiotic Nystatin, which interacts with membrane sterols, increases water, electrolyte and non-electrolyte permeability of cholesterol-lipid bilayer and causes the formation of pores with a radius of 0.36-0.37 nm. Nystatin (SIGMA-ALDRICH) was dissolved in dimethyl sulfoxide. Incubation of washed erythrocytes with nystatin(2.10⁻⁶-10⁻³)M was carried out in 30 minutes at 25°C. The final solvent concentration in the test medium was less than 0.01% [9]. Experiments were conducted with a suspension of erythrocytes after removal of the supernatant. The data are expressed as mean ±SD. Comparisons between the deformability indexes of red blood cell treated with and without drugs were made by paired T-test. P values <0.05 were considered significant.

Results

These experiments were presented in Figures 1 and 2. There was a clear dose-dependent increase of both the indicator Iₘᵢₙ and the Iₘᵢₙ depending on the number of pores formed in erythrocyte membranes(Figure 1), whereas Figure 2 showed a dose-dependent decrease in the ability of red blood cells to deform both at isotropic swelling and in isotonic zone depending on the number of blocked pores. Numerical values of the deformability index Iₘᵢₙ as result of the impacts with nystatin and mercuric chloride were shown in Table 1. As was seen from the table, Iₘᵢₙ significantly increased when increasing the number of hydrophilic pores in the membrane of erythrocyte and start falling at their blockade. Numerical values of Iₘᵢₙ in isotonic region were shown in Table 2. Integral deformability Iₘᵢₙ was...
increased significantly with increase in the number of hydrophilic pores and decreased with their blockade. These concentrations of reagents did not destroy the erythrocyte membranes and have demonstrated the picture of quality changes in deformation properties of red blood cells in model experiments.

![Figure 1](image1.png)

**Figure 1:** Osmotic deformability profiles of erythrocytes before and after incubation with nystatin. Curves 2-6 represent samples with a progressive increase in the concentration of nystatin: 1 - control, 2-1 µM, 3-2 µM, 4-5 µM, 5-10 µM, 6-20 µM.

![Figure 2](image2.png)

**Figure 2:** Osmotic deformability profiles of erythrocytes before and after incubation with HgCl₂. Curves 2-4 represent samples with a progressive increase in the concentration of HgCl₂: 1 - control, 2-20 µM, 3-50 µM, 4-100 µM.

| Nystatin    | HgCl₂   |
|-------------|---------|
| Control, n=5| 0.167±0.008 | Control, n=5 | 0.102 ± 0.007 |
| 1 µM, n=5   | 0.187±0.008 | 20 µM, n=5  | 0.088 ± 0.005 |
| 2 µM, n=5   | 0.190±0.008 | 50 µM, n=5  | 0.081 ± 0.005* |
| 5 µM, n=5   | 0.200 ± 0.008* | 100 µM, n=5 | 0.063 ± 0.004*** |
| 10 µM, n=5  | 0.212 ± 0.009*** |
| 20 µM, n=5  | 0.219 ± 0.010** |

**Table 1:** I_{min} values in experiments with nystatin and HgCl₂. *-P<0.05; **-P<0.01; ***- P<0.001. Note: The reliability of differences in relation to the control.

| Nystatin    | HgCl₂   |
|-------------|---------|
| Control, n=5| 0.575 ± 0.007 | Control, n=5 | 0.528 ± 0.010 |
| 1 µM, n=5   | 0.588 ± 0.008* | 20 µM, n=5  | 0.517 ± 0.008* |
| 2 µM, n=5   | 0.617 ± 0.009*** | 50 µM, n=5  | 0.502 ± 0.008** |
| 5 µM, n=5   | 0.613 ± 0.012*** | 100 µM, n=5 | 0.475 ± 0.006*** |
| 10 µM, n=5  | 0.612 ± 0.008*** |
| 20 µM, n=5  | 0.608 ± 0.010** |

**Table 2:** I_y values in experiments with nystatin and HgCl₂

**Discussion**

Biological membranes form extended bulk bilayer structures with a relatively small microviscosity and thickness combining the protein and lipid components with different properties. Barrier and mechanical properties of the cell are defined its continuity. Membrane lipids possessing mesomorphism reside in the crystalline and liquid states, which differ in packing density and mobility of the protein molecules. The phase transitions lead to an increase in mobility of the acyl chains in bilayer, to an increase their angle of inclination and to a reducing of packing density. The lateral mobility of membrane proteins is increased, increasing the likelihood of their associates. The native structure of the bilayer can be broken in the process of life with the formation of structural defects. The water permeability of membranes is very high. It is assumed that it can pass through the temporary structural defects formed during thermal vibrations tails of fatty acids. These defects (kinks) provide the ability to move across the membrane not only water, but also other small hydrophilic molecules (oxygen, carbon dioxide).

When red blood cell is placed in a hypopsmotic conditions, water rushes into the cell by concentration gradient, the volume increases, and it takes the form of an isotropic sphere before hemolytic stage. Fundamentally at this point deformation properties of the membrane does not play a significant role, erythrocytes is undeformable structure. However, a shear stress in the Couette cell tends to change the spherical shape. While maintaining the volume, the change of the form can occur as a result of increase in surface area only, because sphere has a maximum volume for the given surface. But the extension module (dilatation), determines the properties of the lipid bilayer as a two-dimensional incompressible fluid is so large that for all non-

Citation: Katiukhin LN (2014) About a Mechanism of the Fåhraeus-Lindquist-Effect. J Blood Disorders Transf 5: 1000211. doi: 10.4172/2155-9864.1000211
destructive deformation of the erythrocyte surface area remains unchanged, and the membrane under physiological conditions inextensible [10]. The shear forces cause the rise of the hydrostatic pressure. The volume is reduced due to output a liquid suspension through hydrophilic pores. Thus, the erythrocyte has an ability to change its shape in shear flow due to the exchange of the liquid phases between of its content and suspending medium (in point O). The extent of these changes depends on the number of the liquid phase output from the erythrocyte, i.e. on the number of the liquid pores.

The method developers [11] in the study of cell populations isolated by density gradient have shown that not all cells reach a critical volume in the same osmolarity. Accordingly, they explain residual deformability of erythrocytes in terms of polymorphism. However, the authors did not consider the possibility of exchanging liquid phases in the deformation of cells. In our opinion the erythrocyte polymorphism modifies the width of the inversion zone only and to a lesser extent the residual deformability. Meanwhile, in anemic states, particularly in sickle cell anemia, ektacytometry shows the perversive behavior of osmoskanes, especially in point O."max(12-15). Our years of research suggests that at the native state the deformability in the inversion point is significantly lower than when various impacts, whether pathology or stress.

As seen from the figures, the deformability in inversion point (I"min) increases with the concentration of the antibiotic in the suspension medium and is reduced when using a blocking agent. However, the osmoskane picture also is changing. Thus, the formation of additional pores in membranes (see Figure 1) is violated native osmoregulation and the shape of erythrocytes. The hydration of hemoglobin is increased due to water ingress into the cell. Point O"min is shifted into hyperosmotic zone, indicating that the erythrocytes are swelling. As for the shift point Omaks, this is a consequence of increase in the degree of a hemoglobin hydration and shift of characteristic point "O'of right wing of the osmoskane. When the pores are blocked (see Figure 2), there is a "conservation" of the inner aqueous phase and the osmskane profile does not change. However, the membrane is "loaded" with heavy metal salt and becomes rigid, as evidenced the O"max reduction.

The data of these experiments are shown in a narrow range of concentrations of mercuric chloride and nystatin with minimally noticeable effect. The concentrations of reagents used in the paper do not destroy the membrane and demonstrate the picture quality changes of deformation properties of red blood cells in model experiments. Increasing the concentrations, according to a qualitative change in the osmoskane pattern, causes severe disturbance of osmoregulation systems and marked hemolysis.

**Conclusion**

Evolving shear stress in vessels smaller than 150 microns causes stimulated reshaping oxygen carriers. As a consequence of these changes, the liquid phase is moved by the pressure gradient from the capillary lumen into the erythrocyte. The hematocrit and the blood viscosity in the vessel are reduced. These transformations are reversible. When the erythrocyte leaves capillary, the shear deformations are reduced, cell shape is restored and the water re-enters inside. Using labeled media and fluorescent dyes, as well as experiments with cooking buffers on heavy water and subsequent stress by passing the erythrocyte suspension through a Millipore filters or by syringe hopefully confirms our conclusion.

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