

Alteration of the Erythrocyte's Resistance in Rats with Experimental Diabetes

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Abstract

In chronic hyperglycemia conditions, the structural-functional changes in erythrocytes develop - change the most important abilities of erythrocytes, deformability, and rigidity. These alterations must have a major impact on blood rheology and therefore play the important role in diabetic complications. The aim of the present study was to investigate deformability and osmotic resistance of erythrocytes at different stages of experimental alloxan-induced diabetes. Diabetes in Wistar rats was induced by a single intraperitoneally injection of 12% aqueous alloxan solution (at a dose of 200 mg/kg). The rats with blood glucose levels above 250 mg/dl on the 2d day after alloxan administration were included in the study. The erythrocytes resistance (deformability and osmotic resistance) and oxidative stress intensity according to the lipid peroxidation product, malondialdehyde (MDA), content in blood plasma were determined after 1, 3, 5, 15, and 30 days of alloxan administration. Study results show that osmotic resistance of diabetic erythrocytes was significantly reduced (namely hemolysis rate increased and the spherulisation time decreased) compared to the norm. The intensification of erythrocytes hemolysis coincided in time with the maximal level of glucose and MD in the animals' blood, which indicates, that dysfunction of membrane pumps and disturbance in osmotic balance in erythrocytes develop with the participation of both mechanisms, glycation oxidation of membrane proteins. Changes in erythrocyte deformability develop later, after 5-15 days from the beginning of the observation, which indicates the involvement in this process of other later mechanisms, including further irreversible modification of membrane and cytoplasmic proteins. For the establishment of the molecular basis of these mechanisms and ways of their correction the further studies are needed.

Keywords: diabetes; erythrocytes resistance; deformability; oxidative stress; microcirculation

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1. Introduction

Over 422 million people worldwide are affected by diabetes which is directly responsible for 1.6 million deaths each year [1]. Currently, about 10.3% of the adult population in Europe is estimated to have diabetes, and, as expected, this percentage is going to increase substantially by 2030 due to the fact of obesity and aging [2].

Diabetes remains one of the most acute problems of mankind. There are still unresolved issues in the pathogenesis of diabetes. One such issue is the microcirculatory and metabolic disorders during diabetes mellitus, leading to the development of angiopathies.

In diabetes mellitus, the first to respond to an increase in plasma glucose levels are cells that can absorb glucose even without insulin - vascular endothelial cells and erythrocytes. During their 120 days life span, human erythrocytes are constantly exposed to glucose and other active compounds (oxidants) present in the blood, and therefore they play an important role in different physiological and pathophysiological conditions in the body. In chronic hyperglycemia conditions, the structural-functional changes in erythrocytes develop - change the most important abilities of erythrocytes, deformability, and rigidity. These alterations must have a major impact on blood rheology and therefore play the important role in diabetic complications - the development of diabetic angiopathy.

The aim of the present study was to investigate deformability and osmotic resistance of erythrocytes at different stages of experimental alloxan-induced diabetes.

2. Material and Methods

A total of sixty Wistar male rats (280-350 g) were used in the study. The animals were cared for and used under the Laboratory Animal Research (ILAR) guidelines for the care and use of animals in experimental studies; animals were housed in a well-ventilated room under standard laboratory conditions (12:12 h dark/light cycle). Animals were acclimatized for 3 weeks during which they had free access to a commercial pellet diet and water ad libitum. All animal procedures were approved by the Animal Care and Use Committee of the Tbilisi State Medical University.

2.1. Modeling of diabetes

After the acclimatization period, diabetes in rats was induced by a single intraperitoneally injection of freshly prepared 12% aqueous alloxan solution (at a dose of 200 mg/kg). Control rats (6 rats) received a similar volume of physiological saline. The glucose level was measured after 1, 2, 3, 10, 15, and 30 days of alloxan administration. The rats with blood glucose levels above 250 mg/dl on the 2d day after alloxan administration (90% of the total amount of animals (54 rats)) were included in the study.

2.2. Study of erythrocytes resistance

The deformability and osmotic resistance of erythrocytes were determined after 1, 3, 5, 15, and 30 days of

alloxan administration. The deformability of erythrocytes was determined by the computer filtration-photometric method according to their filtration time in the filter, inversely proportional to the deformability. The osmotic resistance of erythrocytes was studied on the basis of the kinetics of their lysis, which was determined by a highly sensitive photo colorimetric differential method. Erythrocytes, after washing in an isotonic solution, were suspended at 0.5% hematocrit (isotonic solution) and then centrifuged (25°C, 1200g, 5 min) and resuspended in a 0.7% v/v NaCl solution at 0.05% hematocrit. Hemoglobin absorbance was measured at 405 nm wavelength at different time incubation intervals (5 - 200 min. of incubation) [3]. The main parameters of erythrocytes' osmotic resistance (T - time of maximum hemolysis of erythrocytes (period of spherulisation), and t - time elapsed from the introduction of hemolytic agent to the beginning of hemolysis (hemolysis onset time), 1/t - hemolysis rate) were determined by the erythrocyte lysis curve.

2.3. Study of the oxidative stress intensity

The oxidative stress intensity in the animal's blood was studied according to the lipid peroxidation product malondialdehyde (MDA), content in blood plasma. MDA content in blood plasma was determined by Thiobarbituric acid assay [4].

2.4. Statistical Analysis

The obtained data were statistically processed using the student t criterion. Statistically significant differences between parameters were assumed at $p < 0.05$.

3. Results

Fig.1 provides data on the glucose level in the rats' blood at different stages of alloxan-induced diabetes. On the 2nd day of alloxan administration, the blood glucose level in rats increased by 150%, on the 3rd day of observation its level reached a maximum (275% of the control level), and after it began to decrease and on the 30th day of observation reached the control level (Fig. 1).

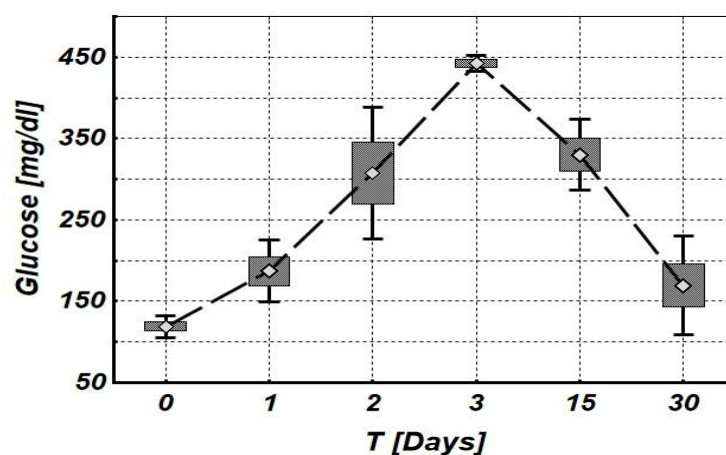


Figure 1: Dynamics of blood glucose indices in rats in the model of alloxan-induced diabetes

Figure 2 shows the dynamics of osmotic resistance of erythrocytes from rats with alloxan-induced diabetes after 1, 3, 5, 15, 30 days after alloxan injection, reported as absorbance of hemoglobin released at different incubation times (5-200 min).

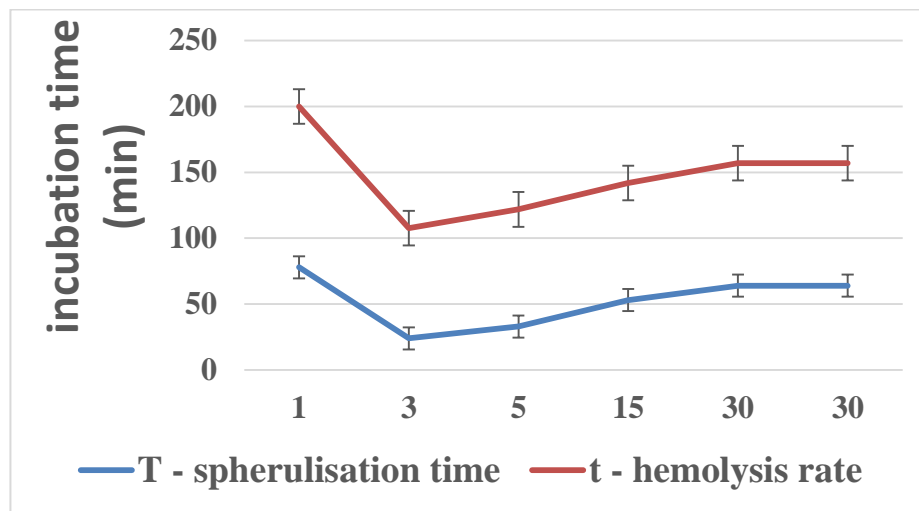


Figure 2: Dynamics of rats' blood erythrocytes osmotic resistance in the model of alloxan-induced diabetes reported as absorbance of hemoglobin released at different times (5-200 min) of incubation.

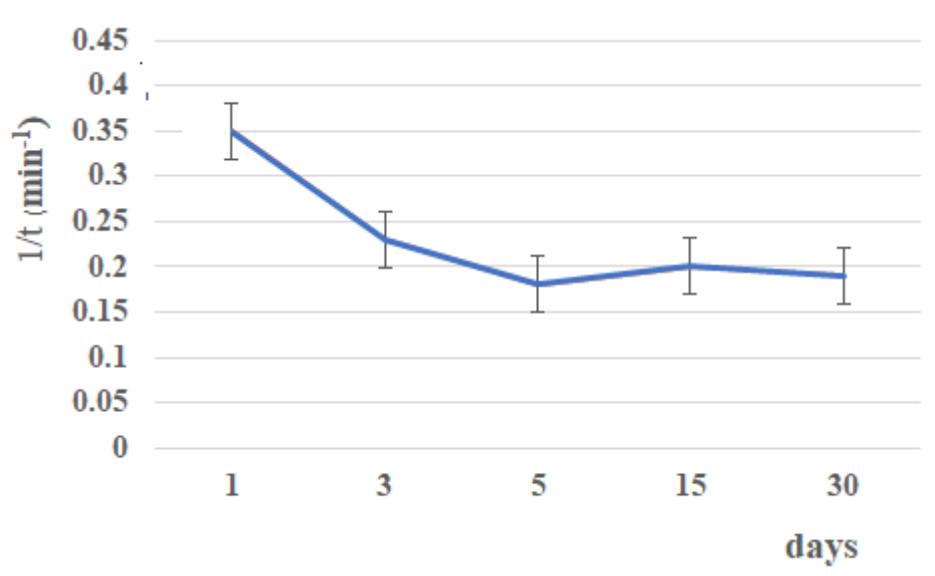


Figure 3: Dynamics of rats' blood erythrocytes deformability in the model of alloxan-induced diabetes reported in terms of the reciprocal of the absorption time of erythrocytes by the filter

As can be seen from the data, on the 3rd day after alloxan administration, the erythrocytes' hemolysis rate (t) and spherulisation time (T) were reduced compared to control values, these parameters increased to the 15th day of the experiment. On day 30th of the experiment, there was a tendency to improve in the erythrocyte's osmotic resistance characteristics, although their osmotic resistance was still significantly lower relative to the

norm, namely, the hemolysis rate (t) was reduced by 22% and the spherulisation time (T) by 18%. Therefore, the osmotic resistance of erythrocytes during diabetes decreases compared to the norm.

Figure 3 presents data on the erythrocyte deformability of rats with alloxan-induced diabetes, which is reported in terms of the reciprocal of the absorption time of erythrocytes by the filter. According to the results of the experiment, on the 3 days after alloxan injection, the erythrocytes deformability was significantly reduced (by 35%), on the 5th day continued to decrease, and reached 49% of initial level and stay at this level to the end of the observation.

A high level of MDA in the blood serum of experimental rats with alloxan-induced diabetes was detected (maximal on the 3d day pf alloxan injection) that indicates the intensification of the oxidative stress and membrane lipid peroxidation processes in the animals' body) (Fig. 4).

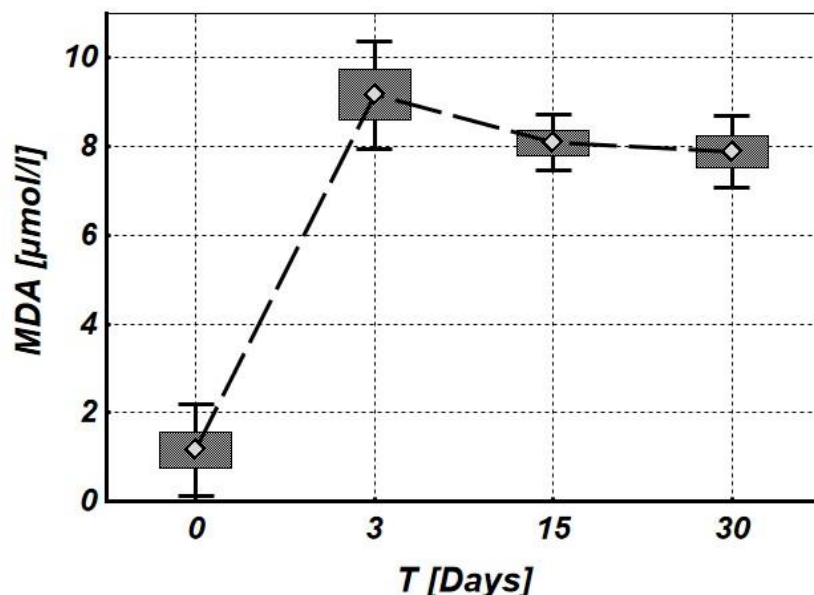


Figure 4: Dynamics of blood MDA indices in rats in the model of alloxan-induced diabetes

4. Discussion

The physical properties (deformability, resistance) of erythrocytes play a crucial role in the maintenance of normal blood flow in microcirculation (1, 5) under physiological and pathophysiological conditions [5]; the precise molecular mechanisms of their alteration are still unclear. Erythrocytes deformability is related to the viscoelastic properties of their membrane, the internal viscosity, and the surface area-to-volume ratio [6].

Viscoelastic properties of erythrocytes membrane depend on the membrane phospholipids (saturated/non-saturated) and cholesterol content, cytoskeleton composition, protein-to-protein interactions. The internal viscosity of erythrocytes depends on cells' volume and property of hemoglobin. Changes in the hemoglobin properties (light globin chains (thalassemia), flexibility (Sickle-cell anemia), etc.), and its concentration in erythrocytes can cause the alterations of their internal viscosity. The changes in the volume, the diameter of

erythrocytes may be caused by disorders of the osmotic pressure balance between intra- and extracellular spaces, due to the dysfunction of the membrane ionic channels, which can subsequently lead to cell hemolysis.

During their life span, erythrocytes of alloxan-injected rats were constantly exposed to glucose and other oxidant compounds; glucose metabolites leading to oxidative stress and lipids peroxidation, causing altered membrane lipid-protein and protein-protein interactions, and finally consequent eryptosis [7]. Lipid peroxidation, protein glycation, and the effects of oxidative stress on proteins are the main possible factors affecting the erythrocyte membrane's elasticity, viscosity, and fluidity during diabetes [8]. It was suggested that decreased activity in the erythrocytes' membrane pumps, related to glycation or oxidative damage of membrane proteins might lead to abnormal electrolyte levels in the erythrocytes and disorders in osmotic balance [7, 9]. Studies have shown a decrease in the activity of Na^+ / K^+ , Ca^{2+} pumps of the erythrocyte membrane in the elderly and patients with diabetes compared to controls, accompanied by changes in Ca^{2+} homeostasis and sodium accumulation inside the erythrocytes [5, 8].

Our study results show that diabetic erythrocytes' osmotic resistance was significantly reduced (namely hemolysis rate increased and the spherulisation time decreased) compared to the norm. The intensification of erythrocytes hemolysis (a decrease in osmotic resistance) in rats with alloxan-induced diabetes coincides in time with the maximal level of glucose and MD in the animals' blood (Fig. 1, 2, 3), which indicates, that dysfunction of membrane pumps and disturbance in osmotic balance in erythrocytes develop with the participation of both mechanisms, glycation oxidation of membrane proteins.

According to the results of our studies, changes in erythrocyte deformability develop later, after 5-15 days from the beginning of the observation, which indicates the involvement in this process of other later mechanisms, including further irreversible modification of membrane and cytoplasmic proteins.

5. Conclusion

The alterations of the osmotic resistance and deformability in the erythrocytes during diabetes develop with the participation of both, proteins glycation and oxidation mechanisms. For the establishment of the molecular basis of these mechanisms and ways of their correction the further studies are needed.

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