Short Communication

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2024.54.008563

Induction of Red Blood Cells Hemolysis and Methods of its Correction

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ARTICLE INFO

Received: January 03, 2023

Published: January 11, 2024

Citation: EV Husakouskaya, TI Filistovich, MS Yermakovich and AA Spadnikaila. Induction of Red Blood Cells Hemolysis and Methods of its Correction. Biomed J Sci & Tech Res 54(3)-2024. BJSTR. MS.ID.008563.

ABSTRACT

Objective of the Study: To study the literature sources about induction of red blood cells hemolysis and methods of its correction in various pathologies.

Materials and Methods: Search, study and analysis of literature data.

Results and Conclusion: Numerous data available in the literature on the induction of red blood cells hemolysis and its correction in various pathologies indicate the need to maintain normal structure and functional properties of RBC as the most numerous and important blood cells by selection of the most optimal methods of hemolysis prevention and correction.

Keywords: Osmotic Resistance; Erythrocytes; Oxidative Stress

Abbreviations: RBC: Red Blood Cells; MPO: Myeloperoxidase; Hemi-MPO: Monomeric MPO; EGCG: Epigallocatechin Gallate; CHF: Chronic Heart Failure

Introduction

Erythrocytes are one of the most sensitive indicators of increased reactive oxygen species exposure. For this reason, the determination of the acid resistance of erythrocytes to haemolytic reagents is of importance to estimate the severity of course of the pathology and efficiency of its treatment. Determining osmotic resistance and studying the biomechanical properties of red blood cells (RBC), due to their unique deformability, is especially crucial for the pathological analysis of hematological diseases. Incubation of human peripheral blood erythrocytes in order to study their osmotic resistance can be carried out in iso- and hypoosmotic solutions (pure saline solution, saline solution with potassium and calcium, phosphate solution, buffered saline solution) by use of flow cytometry. As osmolality decreases in all media and samples, RBC sizes increase [1]. Generation of RBCs ex vivo is an attractive tool in basic research and for replacement of blood components from donated volunteers, therefore the quality of the membrane is of paramount importance as a necessary condition for the survival of RBCs during storage, as well as in the bloodstream [2].

Materials and Methods

Search, study and analysis of data from literature sources about erythrocytes resistance to haemolytic reagents and application of antihemolytic substances in various pathologies in clinical practice and in experiment were carried out.

Results Induction of Red Blood Cells Hemolysis

Hemolysis of RBC can be achieved by various means of action. One of them is the use of bacterial lipopolysaccharide. For this reason, Kurhaluk N [3] proposed to determine the acid resistance of RBS to hemolytic reagents in a general model of inflammation and oxidative stress induced by low doses of bacterial lipopolysaccharides, a single dose of 150 μg [3]. As a result, scientists found damage to RBC membranes and an increase in the percentage of hemolyzed RBCs along with depletion of white blood cells, increased lipid peroxidation due to an increase in malondialdehyde and conjugated dienes, a decrease in antioxidant protection and an increase in the concentration of ceruloplasmin as an acute phase protein. The direct physicochemical interaction of lipopolysaccharide with erythrocyte membranes was

also proven *in vitro* by Brauckmann S, et al. [4] in patients with sepsis leading to hemolysis [4]. Direct action of endotoxin can affect the resistance of rabbit RBCs with endotoxic shock to hydrochloric acid, saponins and osmotic stretch, which the authors associate with impaired permeability and activation of membrane lipid peroxidation [5]. In addition, hemolysis of RBCs can be also achieved under the influence of α-hemolysin produced by the Staphylococcus aureus strain NCTC 5655 [6].

It is important that in purulent-inflammatory diseases, tests of erythrocyte resistance in a hypotonic solution of sodium chloride and at various concentrations of urea in an isotonic environment can serve as criteria for intoxication [7]. It's also interesting that during inflammation, native myeloperoxidase (MPO), a homodimer enzyme that produces an oxidant stored in azurophilic granules of neutrophils, after disulfide cleavage can lead to the formation of monomeric MPO (hemi-MPO) [8]. Both dimeric MPO and hemi-MPO bind to glycophorins A/B and band 3 protein on the erythrocyte membrane, which leads to significant changes in cell volume, morphology, ion channel conductance of the erythrocyte plasma membrane, reduces cell elasticity and cell resistance to osmotic and acid exposure. However, the effect of hemi-MPO on the studied parameters of erythrocytes is lower than the effect of dimeric MPO. Thus, the appearance of hemi-MPO in the blood during inflammation may serve as a regulatory mechanism aimed at reducing the disturbances in the erythrocyte response induced by dimeric MPO. In acute diffuse peritonitis, which is also one of the inflammatory processes and is localized in the abdominal cavity, the use of lectin led to the formation of an additional peak of erythrocyte osmolysis on the kinetic curves and an increase in the deformation of erythrocytes [9]. At the same time, with a favorable outcome of peritonitis, the peak of osmolysis decreased or disappeared, and the deformation of erythrocytes also disappeared, which is important for the diagnosis and prognosis of diffuse peritonitis. The development of peritonitis in an experimental model with intraperitoneal administration of sodium thioglycolate was also accompanied by a decrease in the osmotic resistance of erythrocytes [10].

It's known that elevated cytosolic Ca2+ concentrations activate Gardos K+ channels in human RBC with membrane hyperpolarization, efflux of K+, Cl^-, and osmotically coupled H2O, resulting in cell contraction (Gardos effect). RBCs from K(Ca)3.1-deficient mice (K(Ca)3.1(-/-)) lacked Gardos channel activity and the Gardos effect, but erythrocyte osmotic resistance didn't differ between K(Ca)3.1(-/-) and their wild-type littermates, indicating low or absent Gardos channel activity in unstressed RBC [11]. Under conditions of developing oxidative stress, Ca2+ influx and phospholipid scrambling were significantly less pronounced in K(Ca)3.1(-/-) than in wild-type erythrocytes. At the same time, *in vitro* use of Staphylococcus aureus α-toxin, which forms pores in the cell membrane, led to significantly stronger hemolysis of K(Ca)3.1(-/-) than in wild-type RBC. Thus, the activity of the K(Ca)3.1 channel and the Gardos effect prevent the hemolysis of damaged RBCs, reducing the release of hemoglobin into the circulating blood [11].

Not only the models of inflammation can serve as examples for induction of RBCs lysis. Diabetes mellitus as classic model is also characterized by oxidative tissue damage and impaired microcirculation, as well as deterioration of the RBC properties [12]. A study of the relationship between RBC distribution width (RDW) and their osmotic stability in people of both sexes, without diabetes mellitus and in those with type 2 diabetes based on changes in the concentration of saline solution capable of determining hypoosmotic lysis revealed an interesting pattern [13]. Higher RDW and lower serum iron were found in the diabetic group compared to non-diabetic subjects. An increase in RDW in both women and men with type 2 diabetes mellitus was associated with a decrease in serum iron levels. Anabnormal increase in the content of membrane tubulin leads to partial inhibition of some P-ATPase activities, causing a decrease in cell deformability and their osmotic resistance, which may be important in the development of both diabetes mellitus and hypertension [14].

With regard to the body's adaptation to hemolysis of erythrocytes, it should be taken into account that when exposed to an oxygen atmosphere at moderate pressure (1.10-1.15 atm) for 4 hours, a significant, transient and reversible decrease in the hemolytic resistance of peripheral blood erythrocytes is observed. Two days after oxygen exposure, the bone marrow, as compensation for oxygen damage, releases young RBCs with increased hemolytic resistance, which allows us to consider normobaric oxygen load as a stress factor of moderate intensity [15]. It should also be remembered that the stability of blood samples is critical to the accuracy of determination of osmotic resistance of RBCs. In older blood samples (with lysed cells), markedly different nonlinear behavior is observed due to the presence of free hemoglobin [16]. In addition, whole blood samples should not be refrigerated at 4°C for >2 days before testing [17].

Correction of Hemolysis

As well as knowledge about the induction of hemolysis, the administration of various antihemolytic substances is of importance and can play a crucial role in the treatment of numerous pathologies, including different experimental models. For example, administration of melatonin to animals exposed to bacterial LPS reduces damage to erythrocyte membranes and reduces overall oxidative stress in the plasma, which leads to a significantly higher acid resistance of erythrocytes compared to the reaction of erythrocytes of control mice [3]. The preliminary introduction of the Pro-Gly-Pro peptide in experimental peritonitis led to an increase in the osmotic resistance of erythrocytes and a decrease in the percentage of hemolyzed cells. However, the authors inform that introduction of the peptide after 1 hour 45 minutes of peritonitis didn't have a corrective effect on osmotic resistance [10]. Antihemolytic activity was found in flavonoids. Lipophilic flavonoid quercetin effectively inhibited the effects of α-he-

molysin, produced by the Staphylococcus aureus strain NCTC 5655, resulting in increased the diameter of bacterial cells and increased the fluidity of their membranes, and also increased the rigidity of the hydrophobic region and fluidity of RBC membranes. It follows from this that the antibacterial activity of flavonoids is due to a violation of the structural organization of bacterial cell membranes, and the antihemolytic effect of quercetin is associated with influence of the flavonoid on organization of RBC membrane by preventing the incorporation of the toxin into target membrane [6].

The positive effect of quercetin (20 mg/kg/day for 6 weeks) was revealed in rats with diabetes mellitus in relation to properties of RBC and it suggests a potential age dependence of the effects which were more beneficial in old obese rats with diabetes [12]. Extracts of raspberry (Rubus idaeus L.) and strawberry (Fragaria vesca L.) leaves aren't toxic to RBCs and vascular endothelial cells, but effectively protect cells and their membranes from oxidative damage, therefore the tested extracts can potentially be used to slow down the aging process of the body and prevent many diseases [18]. Extracts of Uncaria tomentosa (Willd.) DC, a woody climbing species native to South and Central America and used in the treatment of asthma, rheumatism, hypertension and for blood purification, change the shape of human RBCs, which may be due to integration into the outer hydrophilic monolayer of the RBC membrane, thereby protecting RBCs from the adverse effects of oxidative stress [19]. In addition, the catechins (+)-catechin, (-)-epigallocatechin (EGC) and (-)-epigallocatechin gallate (EGCG) have been found to have a protective effect against oxidative damage to RBC [20]. The accumulation of catechins in the erythrocyte membrane is characterized by an increase in osmotic resistance and rigidization of the erythrocyte membrane; (-)-epigallocatechin and (-)-epigallocatechin gallate inhibit erythrocyte acetylcholinesterase. Catechins protect erythrocytes from permanganate-induced hemolysis, oxidation of thiol groups of erythrocyte proteins, as well as peroxidation of membrane lipids. These results contribute to the understanding of the beneficial effects of catechins present in plant-based foods and beverages. Vitamin D deficiency (total 25(OH)D) leads to an increase in the number of reversibly and irreversibly deformed RBCs in the blood and a decrease in their osmotic resistance in patients with chronic heart failure (CHF IIA stages II and III functional class) during physical activity (test 6-minute walk), which could potentially have negative consequences for physical health and indicates the importance of maintaining normal levels of this vitamin in this group of patients [21].

In addition to the cytoskeleton and embedded proteins, the lipid bilayer is critical for membrane integrity. High-resolution mass spectrometric analysis revealed a significant deficiency of cholesterol in erythrocytes, which, with minimal fluctuations in osmotic conditions, leads to increased hemolysis of erythrocytes [2]. Additional administration of lipids, especially cholesterol, during cultivation leads to an increase in the cholesterol content of erythrocytes to subnormal levels and restoration of osmotic resistance, which indicates cholesterol deficiency as the cause of increased fragility of erythrocytes. The use of liposomal technologies in order to increase the effectiveness of treatment and reduce its side effects leads to an additional effect – the interaction of liposomes with RBCs during intravenous administration of liposomal dosage forms, which result in a significant change in the transmembrane potential, rigidity and shape of RBCs [22]. These changes are manifested in a decrease in the severity of anisocytosis under conditions of intravenous administration of nano preparations in the liposomal form of phosphatidylcholine, which indicates their beneficial effect on the survival time of erythrocytes. Photobiomodulation has gained widespread recognition for its anti-inflammatory and cytoprotective potential. The beneficial effects of photobiomodulation on cellular function are mainly due to the absorption of red and near-infrared radiation by chromophores such as cytochrome c oxidase. In addition, the study of the effect of photobiomodulation on cells that don't contain mitochondria, for example, erythrocytes, is interpreted by most authors as a membrane protective effect on the structure and function of RBC, manifested in increased osmotic resistance, normalization of membrane permeability, decreased free radical oxidation, decreased membrane activity of phospholipase A2 and normalization of viscoelastic properties of RBCs and their deformability index [23]. It was found that RBCs that received photobiomodulation were less prone to hemolysis, as evidenced by a lower amount of free hemoglobin in the plasma, which indicates a decrease in the severity of hemolysis [24].

Low-level laser therapy is widely used in clinical practice to treat various pathologies, which is believed to be associated with an effect on microcirculation, that depends on the state of RBC. Low-level laser therapy had a beneficial effect in the form of a decrease in the number of pathological forms of erythrocytes, restoration of the phase portraits of erythrocytes, an increase in electrophoretic mobility and osmotic resistance, and the number of erythrocytes in the blood was restored in experimental hyperadrenalineemia [25]. It is assumed that the corrective effect of low-level laser therapy is realized both at the cellular level through the effect of laser radiation on the membranes of erythrocytes, and at the systemic level through the activation of stress-releasing systems of the body with subsequent limitation of the inflammatory response.

As known RBCs are the most numerous cells in the body and carry out gas exchange between all tissues. When aggressive cytostatics are introduced into the body, RBC are among the first to encounter them, which can aggravate the severity of anemia in tumors. Using laser diffraction, flow cytometry and confocal microscopy, it was shown that the cytostatic drug paclitaxel, having a targeted effect on cytoskeletal proteins, alone and in combination with carboplatin leads to the most pronounced disorders: loss of control of volume regulation, resistance to osmotic load and stomatocytosis. In contrast to paclitaxel, drugs such as carboplatin, cyclophosphamide and doxorubicin showed sig-

nificantly less cytotoxicity to RBC after short-term exposure, which may be due to the fact that their main target in cancer cells is DNA rather than the cell membrane [26]. In conclusion, it should be added that air pollution and the problem of «urban particulate matterr, which has arisen due to the acceleration of industrialization and urbanization, have become a serious global problem, posing a threat to human health. By oxidizing hemoglobin, «urban particulate matter» can affect oxygen transport function, increase reactive oxygen species and decrease antioxidant function as measured by malonaldehyde, glutathione, and glucose-6-phosphate dehydrogenase. Attachment of «urban particulate matter» to RBC or uptake by them in high concentrations can alter erythrocyte morphology, leading to increased phosphatidylserine exposure in erythrocytes and subsequent clearance by the mononuclear phagocyte system *in vivo* [27].

Conclusion

One of the most important tasks of modern experimental and clinical medicine is the search for new effective agents that correct disorders of microcirculation and associated erythrocyte membranes. Further clinical trials are recommended to provide further evidence of the effectiveness of antihemolytic therapy, which can, when dosed correctly, improve RBC stability and deformability in RBC-related pathologies. Additionally, for critically ill patients, transfusion of RBCs with abnormal morphology and function may have serious clinical consequences, indicating that potential risks should be considered when screening blood donations. The data obtained can be taken into account in the treatment of inflammatory processes, diabetes mellitus and arterial hypertension, to minimize the side effects of anemia during cancer chemotherapy and a number of other pathologies.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2024.54.008563

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