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Erythrocyte as a Therapeutic Target

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Abstract

Erythrocytes are powerful components of blood flow designed to scavenger or deliver nitic oxide (NO) and oxygen to all cells in the body and transport carbon dioxide from them to the lungs. Blood components started to be quantified and erythrocyte blood shapes used as diagnostic and prognostic tools in clinical practice. Erythrocytes have hemorheological, hemostatic and pro or anti-inflammatory properties enlarging their physiological implications in health and disease. As blood component the erythrocytes establish interaction with others white blood cells, platelets, plasma lipoproteins and vascular endothelial cells. The aim of this mini review is highlight the signaling pathway of nitric oxide in which some steps explain the efficacy of some therapeutic drugs already used and could point new targets for further application in inflammatory vascular diseases

Keywords: Erythrocyte; Nitric oxide; Acetylcholinesterase; Forskolin; CD47; Fibrinogen

Abbrevations: AChE: Acetylcholinesterase; NO: Nitric Oxide; RBCs: Red Blood Cells; GIP: Glucose-Insulin-Potassium; PKC: protein kinase C;

Mini Review

More than two centuries ago was discovered in blood the presence of erythrocytes and its vital function as the unique oxygen carrier binding to hemoglobin which molecular and structural characterizations where later described [1-5]. Erythrocytes with different shapes are observed in association with some hemoglobinophaties for example sickle cell disease, or in those resulting from inserted compounds into specific membrane domains [6,7].

Erythrocyte metabolism provides metabolites able to regulate the oxygen affinity for hemoglobin such as 2,3-bisposphoglycerate and others to participate as coenzymes in antioxidant pathways [8]. Several therapeutically drugs cause hemolysis in humans with glucose-6-phospate dehydrogenase null gene [9]. A reducing environment inside of erythrocytes ensures the active form of hemoglobin with its ferric ion and the normal interaction between biomolecules of the membrane bilayer and the proteins of cytoskeleton [10]. However, when erythrocytes show higher pro-oxidant activity contribute to abnormal biorheological functions associated with inflammatory vascular diseases [11,12]. They can be a trigger or a consequence of micro or macro circulation dysfunction. Acquired ability of erythrocytes to combine with partners of hemostatics components generate red thrombus and help the rolling and adhesion of white blood cells to vascular endothelial wall [13,14].

Erythrocytes are enucleated blood components, but are more than sacks of hemoglobin during the semi life of 120 days comporting different signaling pathways in which is included the final stage of apoptosis (eryptosis) that has been evidenced [15,16]. The appearance in plasma of exovesicules enriched with the acetylcholinesterase (AChE) originated from erythrocyte membrane, the phosphatydilserine exposition in the outer membrane of erythrocyte in addition to kinetic changes of the AChE evaluated in older erythrocytes are biomarkers of red blood cells senescence (RBCs) [17,18]. Previously AChE in erythrocytes was evidenced as a biomarker of its membrane integrity [19].

Depending on the degree of endothelium integrity the circulating acetylcholine [ACh) induce vasodilation or vasoconstriction according the amount of nitric oxide (NO) synthesised and released [20,21]. The NO released from endothelial cells and platelets is scavenged by erythrocyte and blood cell free haemoglobin [22].

Erythrocyte membrane AChE is involved in the nitric oxide (NO) signal pathway as evidenced, for the first time, using blood

samples from blood donors in several in vitro studies in the begin of this century [23,24]. No metabolism provides several NO derivative molecules such as nitrite, nitrate, peroxynitrite and S-nitro glutathione (GSNO) behavior the last one as NO reservoir such as S -nitrosohemoglobin [23,24]. The signal transduction pathway mediated by the enzymatic complex form AChE-ACh is coupled to Gαiproein, adenylil cyclase (AC), band3 protein, protein kinase C (PKC) and phosphodiesterase-3 (PDE-3) [24]. The ACh concentration used is below its substrate optimum concentration [25]. The substrate concentration correspondent to the velocity maximum obtained in the bell shape kinetic curve [25,26]. Higher NO efflux occurs under the influence of AChE-Ach complex, in simultaneous with the band3 protein phosphorylation [23,24]. Compounds that inhibit the protein tyrosine kinase or protein tyrosine phosphatase induce inhibition or activation on AChE enzyme activity [24]. Used two types of AChE inhibitors, velnacrine maleate and timolol generate inactive or less active enzyme AChE-inhibitor complexes that impaired NO efflux from erythrocytes in relation to the active form ACh- AChE [23,27].

In patients with open angle glaucoma, over expression of eNOS and nNOS, decreased levels of cGMP (intermediate in NO signaling) and of nitrite (NO metabolite)in aqueous humour and increased erythrocyte AChE activity were described [28-30]. When blood samples from glaucoma patients is incubated in-vitro in presence of timolol, no changes in the NO efflux neither in the GSNO content of erythrocytes were evidenced besides both molecules are in higher concentrations than the normal values obtained in health humans [31]. This study showed that no reinforcement will occur in the amount of nitrogen reactive stress characteristic of glaucoma patients, by timolol application [31].

Insulin resistance can be eliminated in some patients with sepsis by continuous intravenous infusion of insulin in the form of glucose-insulin-potassium (GIP) regimen that improves survival [32,33]. When blood samples from patients with septic shock where incubated in-vitro with insulin increased the amount of GSNO and the concentration of NO inside erythrocytes was maintained between the normal values [34]. A positive association was observed between NO efflux from erythrocyte and perfused vessel density at sub-lingual microcirculation [34]. So the GPI regimen protected from nitrogen reactive stress [34].

When fibrinogenemia is mimicked *in- vitro* NO efflux from erythrocyte increases, in dependence of band3 protein phosphorylation, returning to normal levels when in presence of either ACh or timolol showing dependency of the AChE enzyme conformational states and of the lower levels of cyclic adenosine Monophosphate (cAMP)concentrations [27,35-37]. When the inhibitor of the erythrocyte Casein Kinase 2, (a cytosol protein that phosphorylated band 3 protein), is present in the erythrocytes suspensions at high fibrinogen concentration the NO efflux level is maintained between normal values confirmed its dependence of band 3 de phosphorylating for be rescued by RBCs [38].

Is very interesting that the forskolin, activator of AC enzyme normalize the levels of NO efflux from erythrocytes in in-vitro model of hyper fibrinogenemia, is nowadays used to alleviate patients with glaucoma [39].

As mentioned above glaucoma patient's present increase nitrogen reactive species in aqueous humor and NO efflux from their erythrocytes are higher than healthy humans [28,31]. So, one explanation for the forskolin therapeutic success in glaucoma patient's could result from NO efflux from their erythrocyte be dependent of lower cAMP levels. This make sense because glaucoma is an inflammatory disease where patients have increased levels of fibrinogen which is known its binding to erythrocyte membrane CD47 that by association with Gαiprotein and AC inhibition decreased cAMP concentrations. [37,40,41]. Besides, this is a clue need to be explored.

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