Chapter 7

Erythrocyte Nitric Oxide

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Additional information is available at the end of the chapter

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Abstract

Nitric oxide (NO) is a vasoactive molecule that, by stimulated and functional vascular endothelial cells, is released to the lumen of the vessel and into the surrounding smooth muscle cells. Once in the lumen, NO is captured by red blood cells and scavenged inside through hemoglobin and derived as NO metabolites. The delivery ability of erythrocytes allowing the NO efflux also occurs. Manipulation of NO levels inside the erythrocyte through different external (acetylcholine, acetylcholinesterase inhibitors, fibrinogen and CD47 4N1K peptide) and internal (redox and protein phosphorylation levels) stimuli will be described. The values of NO efflux from the erythrocytes and its association with the data quantified in the hemorheology properties and in clinical parameters obtained from patients with vascular diseases will also be present. The in vivo animal experimental studies highlighting the ability of NO efflux (delivered) from the erythrocytes where is scavenged and its influence in inflammatory and hemorheological responses will be addressed. So, the aim of this chapter is to present the knowledge obtained about the NO signal transduction mechanism in erythrocytes and the association between erythrocyte availability in NO with clinical biomarkers obtained in inflammatory vascular diseases. A final question is raised—namely, could NO be considered a hemorheological parameter?

Keywords: erythrocyte, nitric oxide, deformability, intravital microscopy, pathophysiology

1. Introduction

Vascular endothelium cells behave like "meeting points" between white blood cells and mediator factor participants in the steps of inflammatory response allowing "cross-talk" with blood, red blood cells (RBCs), platelets, fibrinogen, lipoproteins and other blood biomolecule components [1]. Endothelial cells (ECs) under influence of mechanical, physical and chemical stimuli are prone to secrete vasoactive molecules into the lumen vessels and smooth muscle cells [2].

The endothelium-derived factor secreted by vascular endothelial cells, known by its vasodilator property, was further identified as nitric oxide (NO) by Robert F. Furchgott, Louis J. Ignarro and Ferid Murad who received the Nobel Prize in 1998 [3, 4].

Ignarro's spectral analysis of hemoglobin (Hb) evidenced that when this biomolecule was exposed to endothelial cells' stimulate by acetylcholine (ACh), NO is liberated and a shift of the Hb absorption curve occurs, establishing for the first time the NO binding to Hb and an indirect link between red blood cells (RBCs) and NO [4].

Other authors evidenced the ability of RBCs to rescue NO liberated from endothelium cells and the need to liberate it according the tissues oxygen partial pressure [5, 6]. Experiments conducted in vitro using RBCs under normoxia conditions submitting to low oxygen tension (hypoxia) showed liberation of oxygen and NO binding to deoxygenated hemoglobin demonstrating consequently allosteric structure transitions of the Hb molecule [5]. The role of erythrocyte membrane band 3 protein into NO through erythrocyte was studied [5]. The bioavailability of RBCs in NO may be the trigger or the consequence of the involvement of the RBC's hemoglobin as the oxygen sensor [5, 6]. NO binds to oxygenated Hb in its thiol group of cysteine β93 at high-tissue oxygen pressure (PaO_2) originating as S-nitrosohemoglobin (SNO-HbO_2), while at low PaO_2, NO binds to the iron ion of the hemoglobin; heme group generates nitrosylhemoglobin molecules [7, 8]. Regarding the efflux of NO from erythrocytes, the transfer of NO between SNO-HbO_2 and the thiol group of band 3 protein was verified [9–11]. The transnitrosation reaction could occur with the thiol group of other biomolecules [9–11]. Using inhibitors of protein tyrosine kinase (PTK) p72 syk, Src Lyn and of SHP-2 protein tyrosine phosphatase (PTP), in vitro studies have evidenced, respectively, band 3 protein phosphorylation and dephosphorylation at tyrosine residues [12]. The dephosphorylation of band 3 is associated with oxyhemoglobin and glycolytic enzymes binding which, upon band 3 phosphorylation, delivers oxygen and glyceraldehyde dehydrogenase, aldolase and phosphofructokinase closer to the cytosol [13]. Glutathione (GSH) is a redox biomolecule resulting from the reaction between the three peptides glycine, glutamic and cysteine showing its thiol group that can bind directly NO transferred from SNO-HbO_2, originating from nitrosothiol such as S-nitrosoglutathione (GSNO) [14]. The GSNO is a transient reservoir of NO because it is essential to be in its reduced state for the regeneration of NADPH to NADP levels of the erythrocyte. However, the inactivation of glutathione reductase induced by oxidative stress influences the concentration of GSH which is needed for regeneration of oxidized proteins [15]. For instance, dithiothreitol (DTT) is a thiol-reducing agent capable of regenerating disulfide-containing proteins and establishing an interchangeable thiol-disulfide reaction with glutathione [16]. Beyond that, DTT's presence induces changes on enzyme activity states, for example, of the PTP and PTK [17].

If auto-oxidation of hemoglobin occurs the peroxide anion will be produced, which generates peroxynitrite after reaction with NO [18]. The decomposition of peroxynitrite molecules leads to nitrite (NO^{2-}) and nitrate (NO^{3-}) which are designated NO derivative molecules (NO^x) whose concentrations are changed by external RBC-binding biomolecules as shown [19, 20]. It was evidenced that NO release from SNOHb could bind to thiol groups and be exported from RBCs as nitrosothiol or may be so as oxidation generates nitrate [19]. The NO in the presence of oxyhemoglobin molecules induces methemoglobin and nitrate formation [20]. The hemoglobin reductase with the NADH produced in the glycolytic pathway maintains the methemoglobin concentration [21].

Endothelial cells and lymphocytes are able, by the participation of choline acetyltransferase, to synthesize ACh which is released into the plasma through vesicular acetylcholine transporter [22–24].

Depending on the degree of endothelium integrity the circulating ACh induced vasodilation or vasoconstriction according to the amount of nitric oxide synthesized and released [3]. The NO released from endothelial cells and platelets is scavenged by erythrocyte and blood cell-free hemoglobin [25]. In order to identify the signal transduction mechanism of the erythrocyte ability to scavenge or deliver NO, in vitro studies mimicking normal and inflammatory conditions were performed and are presented in the next section. This section also includes ex vivo studies showing the quantification of the NO efflux values, performed with RBCs obtained from blood samples of healthy donors and patients with vascular inflammatory diseases and their association with clinical biomarkers. Also, *in vivo* studies' conduct in animal models of hypertension and inflammation are included in the next section to show erythrocyte NO availability, contribution and association with inflammatory vascular diseases. From all data obtained and herein described we are able to conclude the erythrocyte NO translocation across the erythrocyte membrane as a hemorheological parameter.

2. Erythrocyte nitric oxide studies

2.1. In vitro

Erythrocyte membrane acetylcholinesterase (AChE) is a hydrolytic enzyme with a rare kinetic profile with an optimum substrate concentration (So) from on AChE activity decrease with the augment of its substrate ACh [26, 27]. At lower or higher So values, the AChE-ACh enzyme complex forms are active or the less active ones, respectively [26, 27].

Based on the fact that SNO-Hb and GSNO have been considered reservoirs of NO and ACh is an endogenous compound with vasoactive properties, present in blood circulation, we raised three questions, whether ACh induces changes on erythrocyte deformability, if there is NO inside erythrocyte and whether it could be mobilized to the outside. In order to answer these, human erythrocyte suspensions, in the presence of ACh, were loaded with the permeable non-fluorescent probe diamine fluorescein-2 diacetate (DAF-2 Da). Intra-erythrocyte fluorescence intensity of triazolofluorescein (DAF-2 T) was visualized, by fluorescence microscopy, as a result of the reaction between NO and the 4, 5- diamine fluorescein [28]. So, inside the erythrocyte, there is NO when stimulated with ACh and also there is an increase in the levels of NO^{2-} and NO^{3-} [28]. When erythrocytes are in the presence of ACh, erythrocyte deformability, during the impairment of oxygen hemoglobin affinity and of erythrocyte aggregation (EA), has been verified [29]. The presence of an active complex (AChE-ACh) in red blood cells is able to trigger band 3 protein phosphorylation when PTP is inhibited, with a higher mobilization of NO-derived metabolites [30]. This complex is unable to induce band 3 phosphorylation upon p53/56lyn and p72syk inhibition, providing a lower degree of NO efflux and NOx mobilization [30]. This mobilization is enhanced with phosphorylated but not a dephosphorylated band 3 protein. The maximum translocation of NO efflux from RBC achieved upon acetylcholine stimulation and band 3 phosphorylation was related to the higher levels of the methemoglobin,

[L-lactate], concentration ratio between cyclic guanylyl cyclase (cGMP) and cyclic adenosine monophosphate (cAMP) and lower oxygen affinity to hemoglobin value and of oxyhemoglobin concentration [30]. At variance, the effect of the AChE inhibitor velnacrine maleate (VM) induced a higher degree of [NO] efflux/[NOx] mobilization through the AChE-VM inhibitor complex in the presence of p53/56lyn and p72syk inhibitors [30]. When in the case of erythrocyte membrane band 3 protein dephosphorylated state, the inactive complex form of the AChE promotes higher NO efflux than the AChE active complex form [30]. But the opposite was observed with erythrocyte membrane band 3 protein phosphorylation [30]. When experiments were done with the AChE strong inhibitor, VM, an almost inactive complex, results and induces lower NO efflux from erythrocytes and higher GSNO and peroxynitrite concentration values than those obtained with the active complex form AChE-ACh [30].

Additional studies were performed taking into account the identification of the type of a G-protein involved in the erythrocyte ACh/NO signaling pathway. It was evidenced that at the N-terminal band 3 protein domain only $G_{\alpha i1/2}$ binds. $G_{\alpha i1/2}$ and the G_{β} are associated with band 3 protein at the C-terminal site domain independently of the band 3 phosphorylation degree [31]. This chapter confirmed our previous hypothesis of the potential involvement of a heterotrimeric G protein in signal events mediated by the erythrocyte membrane AChE-ACh complex or AChE-inhibitor complex band 3 protein interactions with the participation of adenylyl cyclase inhibition [20, 31].

The quantification of NO efflux from the erythrocyte was assessed, by the first time for us, using the amino-IV sensor by the amperometric method which is described [32, 33]. The nitric oxide release from RBC in presence of ACh is sense by an electrode which oxidize NO at the working platinum electrode, resulting on electric current. The redox current is proportional to the NO concentration outside the membrane and it was continuously monitored with a computer. The AChE-ACh active complex activates PKC which phosphorylates PTP and PTK switching them to inactive and activate enzymes states, resulting in band-3 protein phosphorylation by PTK active form without with consequently NO release [32–34].

Beyond the AChE's strong inhibitor velnacrine, the moderate AChE inhibitor timolol was used, forming a less active AChE-timolol complex, and a lower erythrocyte efflux from NO was quantified in relation to those values obtained with AChE-ACh [35–37].

For the first time we evidenced that when erythrocytes were in the presence of ACh or timolol, the efflux of GSNO was lower with AChE-timolol than with AChE-ACh, both values being higher than in their absence [38].

It was evidenced by those in vitro studies that AChE's active and less active molecular conformations induce increased or decreased NO efflux from erythrocytes, respectively [38].

In the presence of SpermineNONOate, one among other NO donors, there is an increase in erythrocyte deformability and oxygen hemoglobin affinity (29).

The plasma levels of ACh increase in inflammatory pathologies like fibrinogen (Fib), a plasma molecule predominantly produced by the liver [39, 40]. From many years, it was recognized that Fib behavior in vascular domains, where blood circulation is under low shear stress, acts as the most influent molecule in erythrocyte aggregation (EA) [41]. The association between Fib and EA has been verified in several pathological conditions [42]. Only in this twenty-first

century was CD47 established as a binding target in the erythrocyte membrane for the soluble form of fibrinogen [43].

It was shown that for soluble Fib, in physiological concentrations, the NO efflux from erythrocytes decreased with increased GSNO, nitrite and nitrate levels [44]. The scavenging NO RBC ability to reduce efflux was surpassed showing normal values when both 4N1K (the CD47 peptide analog of thrombospondin binding site) and high fibrinogen levels are present or when 4N1K is absent [45]. These data show the dependence of lower cyclic adenosine monophosphate (cAMP) associated with adenylate cyclase (AC) inhibition by $CD47G_{\alpha i}$ [45]. When phosphorylation of the erythrocyte membrane protein band 3 is induced in the presence of high fibrinogen concentration and in the absence or presence of 4N1K, the NO efflux increases [46, 47]. The NO efflux from erythrocytes at high fibrinogen concentration is dependent on band 3 protein phosphorylation which was confirmed in the experiments where the erythrocyte casein kinase 2 (a cytosol protein that phosphorylates the band 3 protein) inhibitor was used, showing unchanged levels of NO efflux in relation to its absence [27, 48].

During inflammation high levels of both acetylcholine and fibrinogen are presented and normal values of NO efflux from erythrocytes have been observed in vitro [39, 49]. Besides, a higher NO efflux from RBC will be expected resulting of the presence of ACh and high fibrinogen concentration, normal values were obtained; the AChE-ACh molecular conformational state activates PKC which inhibits PDE 3 with increase of cAMP concentration that normalize the lower levels of cAMP derived from the inhibition of AC [35, 38, 45, 49]. Interestingly, the presence of forskolin (activator of the enzyme AC) in an in vitro model of hyperfibrinogenemia did not change the levels of NO efflux from erythrocytes, because the PDE3 is functional to hydrolase cAMP [27, 48].

When stimulating the erythrocyte redox thiol status by loading dithiothreitol (DTT), there is a decreased NO efflux concomitant with increased levels of nitrite, nitrate and GSNO [50]. It is well known that dithiothreitol induces band 3 dephosphorylation and a dephosphorylated state accounts for the AChE inhibitors and fibrinogen effects on red cells [17, 30, 49].

High concentrations of oxidized LDL when in the presence of blood samples of healthy human erythrocytes increase its ability to scavenge NO [51]. The same behavior was obtained in another study conducted with blood samples taken from healthy humans and exposed or not (control aliquot) to two different concentrations of LDL/HDL; no changes in NO efflux values from the erythrocyte, no alterations on intra-erythrocyte peroxynitrite concentrations and an unaltered deformability profile, at all shear stresses, were observed. At variance the levels of intra-erythrocyte NO derivative molecules nitrite, nitrate and GSNO showed significantly increased values when compared with control aliquots. The unchanged deformability values obtained at lower and high shear stresses for all treated blood sample aliquots with LDL/HDL are indicative of membrane stability, internal viscosity maintenance and normal interactions of membrane peripheral and cytoskeleton [52]. The absence of erythrocyte membrane instability obtained in blood sample aliquots under LDL/HDL addition is confirmed by the unchanged nitrogen reactive species concentration of peroxynitrite, as evidence by the normal levels obtained for peroxynitrite is an index of auto-oxidation of oxyhemoglobin [52, 53]. The addition of different concentrations of the lipoprotein sub-fractions' LDL/HDL seems not to favor hemoglobin auto-oxidation. Superoxide anion will be formed from the

auto-oxidation of hemoglobin, but without its generation plus unchanged values of peroxynitrite concentrations it was evidenced that when the thiol status of erythrocyte was maintained in normal range, no alterations were verified in erythrocyte deformability [50].

The NO efflux from erythrocytes is gender independent [54] at variance with higher women's RBC AChE enzyme activity than men as previously evidenced [54]. Timolol maleate is a topically therapeutic drug used in glaucoma patients that, when incubated with blood samples of patients with this nerve optical disease, they did not induce significant differences in NO efflux from erythrocytes and nor in GSNO concentration values inside RBCs when compared to the absence of timolol in the blood aliquots of those erythrocytes [55]. Both NO efflux and GSNO values obtained were significantly higher than those quantified in blood samples of healthy persons [55]. Erythrocytes' NO metabolism in glaucoma patients are not affected by timolol treatment [55].

The same amperometric NO sensor, mentioned above, was used in confluent human umbilical endothelial cells (HUVECs) in which we have demonstrated the existence of membrane-bound acetylcholinesterase and higher NO production in the presence of ACh in relation to velnacrine [56–58]. The activation of the signal transduction mechanism induced by the AChE-ACh active complex revealed high values of [cAMP] and [cGMP] which are lowered by the AChE-VM inactive complex [58].

2.2. Ex vivo

Hemorheology is the science which studies blood deformation and its components' interaction with vessel walls, occurring inside blood vessels of macro and microcirculation. In the past, the longitudinal and follow-up clinical studies done, ex vivo, have as an objective the characterization of the intravascular profile of different diseases according to hemorheological parameters and inflammatory factors [41, 48, 59]. To accomplish this aim, blood samples were taken from patients with acute myocardial infarction, glaucoma, Bechet, renal diseases whether submitted or not to chronic hemodialysis or kidney transplant and diabetic retinopathy degree, and an association between the laboratorial data and the clinical parameter values was observed [41, 48, 59].

Erythrocytes scavenge and liberate oxygen and NO at high and low local tissue oxygen partial pressure, respectively [60, 61]. Erythrocyte deformability is a biorheological influent factor on blood viscosity, cellular oxygenation and a biomarker of acute and chronic inflammation [62].

Patients with hypercholesterolemia, hypertension and renal transplant present lower ability to reversible change its shape (decrease erythrocyte deformability values) and higher values of NO efflux from erythrocytes when stimulated with ACh [63]. In the same study, an inverse relationship between erythrocyte deformability values and NO efflux concentrations from erythrocytes obtained from blood samples of those patients, was evidenced [63]. In all samples lower values of NO efflux were verified in relation to those of healthy persons—what could be considered as a compensatory mechanism to avoid more wall vessel damage [63]. We will present in vivo studies later in this section.

Disturbed blood rheology in patients with systemic lupus erythematosus (SLE) and patients with rheumatoid arthritis (RA) that could contribute to atherosclerosis is described [64–66].

So, a study was performed to evaluate the associations between hemorheology parameters including the erythrocyte NO rescue ability of RBCs and the cardiovascular risk factors, inflammatory parameters and subclinical atherosclerosis. Erythrocyte NO efflux was significantly associated with both carotid intima-media thickness (cIMT) and the presence of plaques (negative association) and was an independent predictor of cIMT [67]. Erythrocyte NO production can be looked at as a compensatory mechanism [67]. As mentioned above, under low tissue oxygen partial tension, erythrocytes release NO bound to hemoglobin, promoting vasodilation [61, 62]. Besides, NO could represent a protective factor against atherosclerosis; it could be produced in large amounts by the inducible nitric oxide synthase (iNOS) which is characteristic of the dysfunctional endothelium which combining with superoxide anion generates peroxynitrite molecules that have oxidant properties; this NO derivative worse the dysfunctional endothelial wall hindering it to return to be functional [68].

The data of the hemorheological and inflammatory evaluations performed ex vivo in blood samples of women with SLE suggested greater risk of arterial thrombosis and prediction of higher mortality than humans with normal blood viscosity and fibrinogen values [67, 69]. Both SLE and RA patients showed high erythrocyte aggregation independent of the medication undertaken by SLE patients. This ex vivo study shows that hemorheological parameters are independently associated with the early stages of atherosclerosis in SLE and RA patients. Additionally, it documents disturbed and unfavorable hemorheological features in association with disease activity and with traditional CV risk factors contributing to atherogenesis in inflammatory rheumatic diseases. So, the evaluation of NO and also of hemorheological parameters must be done in order to predict the development course of the autoimmune disease in RA and SLA patients [67].

In patients with amyotrophic lateral sclerosis (ALS) that is a neurodegenerative disease of the motor system, our aim was to assess RBCs' biochemical and hemorheological parameters and identify novel biomarkers of one of the most painful and fast mortal disease after diagnosis [70]. The erythrocyte deformability and AChE activity of blood samples were increased in patients with ALS in comparison to healthy donors [70]. This ex vivo study conducted with blood samples of ALS patients showed lower values of NO efflux from RBCs and nitrites than those obtained in healthy humans [70]. Due to variability between the duration of this disease until death, the higher NO quartile values are associated with the worse respiratory function [70]. A positive relation of quartiles values were obtained between AChE enzyme activity and nitrites levels [70]. Both erythrocyte NO and AChE were suggested as biomarkers of ALS and further potential therapeutic targets [70].

Another very sad complex situation with high mortality covering healthy humans from all ages and under a variety of situations from a simple infection or travel accident to a post-surgery complication is sepsis [71]. Several pathophysiological mechanisms from unbalanced pro−/anti -inflammatory, hypo and hyper-insulinemia, to hypo- and hyper-glycemia are simultaneously deregulated in intensive care units (ICUs). Follow-up studies carried out in ICU are urgently needed [71]. We have verified that in septic shock patients before dead, at 24 in IUC showed higher efflux of NO from erythrocytes and worse blood circulation observed by hemodynamic parameters namely high unequal blood flow and high microvascular flow index quantified in sub lingual microcirculation [72].

2.3. In vivo studies

The ACh molecule has a ubiquity property with an anti-inflammatory effect, decreasing leukocytes' adherence and plasma TNF-α concentration evidenced in an in vivo animal model of lipopolysaccharide-induced inflammation [73, 74].

The influence of NO on the hemoglobin affinity to oxygen and on biorheology properties of the erythrocytes was shown in healthy and in ill humans as described earlier. NO influx into erythrocytes induced by spermineNONOate or the efflux stimulates by ACh increased the reversible discocyte shape, or erythrocyte deformability, under shear stress values characteristic of the microcirculation network as shown [29].

The angiotensin II AT1-receptor antagonist, valsartan, is able to restore, in hypertensive Sprague–Dawley rats which are LNAME dependent, their systolic blood pressure (SBP) to the physiological values, as well as normalize the whole blood viscosity (WBV) values increased during the hypertensive time [75]. The NO efflux from erythrocytes decreases in parallel to WBV and SBP returning to normal values after valsartan application [75]. Regarding erythrocyte deformability values that decreased during the hypertensive state of the animals, lower or normal values at lower and higher shear stress, respectively, were maintained after systolic blood pressure recovery by valsartan [75]. This in vivo animal experimental model of hypertension demonstrated the relation between the endothelial cells and the erythrocytes' availability of NO, beavering as a compensatory mechanism vascular disturbance [75]. This could be considered as an antagonist effect to the occurrence of reactive nitrogen species (RNS) and to the amplification of oxidative reactive species (ORS).

The in vivo mice model of acute inflammation induced by intra-scrotal injection of the platelet-activator factor (PAF, a phospholipid mediator of inflammation) showed that the NO efflux from erythrocytes decreases with acute-phase response development [76] The end of the acute inflammatory response visualized by intravital microscopy showed normalization of the number of labeled neutrophils rolling and adherent, rolling velocity and vessel diameter values [76]. NO normal values rewound with inflammation recover, besides the maintenance of decreased RBCs' deformability [76]. There are PA receptors (PAF-R) constitutively present on platelets, leukocytes and endothelial cells but are absent in red blood cells; besides, PAF stimulates the breakdown of sphingomyelin on RBCs in isotonic conditions [77]. Therefore, PAF may cause changes in the physicochemical structure of the erythrocyte membrane, which in turn may cause changes in RBC deformability maintained after the recovery of the initial phase of inflammatory response [77]. The NO efflux from erythrocyte behavior in this model of acute inflammatory response during 6 h in mice reinforces the idea of the NO rescue inside erythrocyte as a compensatory mechanism in low-grade or chronic inflammation of those diseases reported earlier [63, 77].

3. Conclusions

The nitric oxide mobilization inside or outside the erythrocyte is possible under either the action of the non-neuronal cholinergic components, acetylcholinesterase and acetylcholine, or the manipulation of band 3 protein phosphorylation degree. Higher NO efflux occurs under the influence of the AChE-ACh complex as well as, simultaneously, with the band 3 protein

phosphorylation. At variance, higher NO mobilization inside the erythrocytes happens under simultaneous influence of AChE-velnacrine and band 3 protein dephosphorylation.

At normal acetylcholine plasma levels, the erythrocyte NO efflux increases by a signal pathway dependent of membrane band 3 protein phosphorylation, $G_{i\alpha\beta}$ protein, AC, acetylcholinesterase enzyme activity and its molecular conformations, PKC and PD3.

The erythrocyte aggregation tendency is impaired by the presence of the AChE-ACh complex and is reinforced by higher thiol redox status inside the erythrocyte. The erythrocyte aggregation is impaired by changes on band 3 phosphorylation/dephosphorylation equilibrium; besides, higher values are associated with a higher phosphorylation degree. On the contrary, ED increases in the presence of the AChE-ACh complex.

The ability of RBC to scavenge NO may be considered as a compensatory mechanism acting against the overproduced NO by the endothelial-inducible NO synthase when the vascular endothelium is dysfunctional [50].

Under unstimulated erythrocytes, GSNO efflux did not occur, and it is regarded as a potentially therapeutical agent, acting as a store or donor of NO.

It is possible to modulate erythrocyte availability in NO by plasma fibrinogen in a nonlinear, dose-dependent manner in human erythrocytes. Lower intra-erythrocyte cAMP is an influent condition to the NO efflux in an in vitro model of hyperfibrinogenemia. These results may be considered a useful therapeutic approach for the storage of blood that is used in transfusions. Fibrinogen-C47-triggered erythrocyte GSNO and decreased NO efflux may, if verified in vivo, be associated with coagulopathy and hypotension under acute phase states. These effects show on NO-derived molecules, allowing intra-erythrocyte NO scavenging as a protector under inflammatory conditions as we evidenced in an animal model of acute inflammation and verified ex vivo in a vascular inflammatory disease with low-grade or chronic inflammation. An anti-reactive nitrogen role can be attributed to ox-LDL for its contribution in the erythrocyte-scavenged ability for nitric oxide. From all studies reviewed here, we can suggest NO efflux or influx from or into RBCs and the internal mobilization between the NOx as a hemorheological parameter participating in the erythrocyte deformability in dependence of the structural protein conformations or phosphorylation degree components of the membrane or the internal RBC compartment.

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Conflict of interest

The authors declare no conflict of interest.

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