https://doi.org/10.46344/JBINO.2025.v14i03.27

HEMOGLOBIN-NITRIC OXIDE INTERACTIONS IN SICKLE CELL DISEASE: MECHANISTIC INSIGHTS

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ABSTRACT

Sickle cell disease (SCD) is a complex hematological disorder marked by chronic hemolysis, inflammation, and recurrent vaso-occlusive crises. A critical but often underemphasized factor contributing to the pathophysiology of SCD is the disruption of nitric oxide (NO) signaling due to excessive interactions with hemoglobin. The release of free hemoglobin into the plasma during hemolysis rapidly scavenges NO, impairing its vasodilatory and anti-inflammatory functions. This biochemical imbalance contributes significantly to endothelial dysfunction and vascular complications observed in SCD patients. The bioavailability of NO is further diminished by increased oxidative stress and the release of arginase from lysed erythrocytes, which depletes L-arginine—an essential substrate for endothelial NO synthesis. The resulting NO-deficient environment promotes vasoconstriction, leukocyte adhesion, and platelet activation, escalating the risk of vascular occlusion and organ damage. These processes not only exacerbate the frequency and severity of vaso-occlusive episodes but also contribute to the long-term morbidity associated with SCD, such as pulmonary hypertension and leg ulcers.

Keywords: *Hemoglobin, Nitric Oxide, Sickle Cell Disease, Vasculopathy, Oxidative Stress*

Introduction

Sickle cell disease (SCD) is a hereditary hemoglobinopathy resulting from a single nucleotide substitution in the β-globin gene, leading to the production of hemoglobin S (HbS). When deoxygenated, HbS undergoes polymerization, causing red blood cells (RBCs) to assume a characteristic sickled shape. These abnormally shaped cells are prone to hemolysis and can obstruct microvasculature, resulting in ischemia-reperfusion injury, chronic pain, and progressive organ dysfunction. While the mechanical consequences of sickled cells have long been central to understanding SCD pathophysiology, growing evidence points to significant contributions from biochemical and molecular dysregulation, particularly involving nitric oxide (NO) bioavailability [1-3]. Nitric oxide is a gaseous signaling molecule synthesized primarily by endothelial nitric oxide synthase (eNOS). It plays a critical role in maintaining vascular homeostasis through vasodilation, inhibition of platelet aggregation, and suppression of endothelial cell activation. In healthy individuals, NO diffuses across cell membranes and acts on vascular smooth muscle cells, promoting relaxation via cyclic guanosine monophosphate (cGMP)-mediated pathways. Its regulatory functions are tightly controlled by local synthesis, availability of substrates such as L-arginine, and interactions with other molecules, including hemoglobin [4-6].

In the context of SCD, intravascular hemolysis is a central event that disrupts NO homeostasis. The rupture of RBCs releases cell-free hemoglobin into the plasma, where it reacts with NO at a rapid rate. This reaction not only reduces the concentration of NO but also generates methemoglobin and nitrate, both of which are biologically inactive. Furthermore, hemolysis leads to the release of erythrocytic arginase, which depletes L-arginine—thereby limiting the substrate required for NO synthesis. The combined effect is a profound reduction in NO bioavailability, contributing to vascular complications that characterize SCD [7-8]. This NO-depleted state initiates and perpetuates endothelial dysfunction, a hallmark of SCD-related vasculopathy. Endothelial cells under reduced NO influence become pro-adhesive and pro-inflammatory, increasing the expression of adhesion molecules such as VCAM-1 and E-selectin. These changes promote the adhesion of sickled RBCs and leukocytes to the vascular wall, further impeding blood flow and escalating the risk of vaso-occlusion. Additionally, reduced NO levels impair the normal anti-thrombotic and anti-proliferative effects on vascular smooth muscle, which may contribute to complications such as pulmonary hypertension [9-10]. Oxidative stress further exacerbates NO dysregulation in SCD. Hemolysis and ischemia-reperfusion injury lead to the generation of reactive oxygen species (ROS), which degrade NO and amplify vascular damage. Free heme, another byproduct of hemolysis, catalyzes oxidative reactions and induces inflammatory signaling cascades. These processes contribute to a vicious cycle in which NO deficiency, oxidative stress, and vascular inflammation mutually reinforce one another, leading to chronic endothelial injury and systemic complications [11-12].

Aim

This review aims to critically explore the mechanistic interplay between hemoglobin and nitric oxide in the context of sickle cell disease, elucidating how their disrupted interaction contributes to vascular dysfunction and disease pathophysiology.

Hemoglobin and Nitric Oxide: Physiological Interactions

Under normal physiological conditions, nitric oxide (NO) serves as a critical mediator of vascular tone and cellular signaling, particularly in the endothelium. Synthesized by endothelial nitric oxide synthase (eNOS) from the amino acid L-arginine, NO diffuses freely across cell membranes and activates soluble guanylate cyclase in adjacent smooth muscle cells, leading to the production of cyclic guanosine monophosphate (cGMP) and resultant vasodilation. Beyond its vasodilatory effects, NO also inhibits platelet aggregation, leukocyte adhesion, and smooth muscle proliferation, all of which are vital to maintaining vascular integrity and function [13-14]. Hemoglobin (Hb), the oxygen-carrying protein in red blood cells, has a complex relationship with NO. Inside intact erythrocytes, the reaction of NO with oxyhemoglobin (HbO_2) is spatially restricted, allowing for a delicate balance between oxygen transport and NO signaling. Under controlled conditions, NO can bind to the heme moiety of hemoglobin or form S-nitrosothiols by interacting with cysteine residues on the globin chain. These interactions serve as part of a physiological reservoir that modulates NO bioactivity and allows for its controlled release, contributing to hypoxic vasodilation—a mechanism by which blood flow is matched to oxygen demand in tissues [15-17]. The protective compartmentalization of hemoglobin within red blood cells ensures that NO is not rapidly scavenged in the bloodstream. This spatial organization prevents premature NO inactivation and preserves its signaling role in the vascular system. However, when hemoglobin escapes into the plasma—such as during hemolysis—this regulatory mechanism is lost. Cell-free hemoglobin in the plasma reacts with NO at an extremely high rate, forming methemoglobin and nitrate. This reaction not only depletes NO but also disrupts vascular homeostasis, leading to vasoconstriction and endothelial dysfunction. Thus, under physiological conditions, hemoglobin functions in a tightly regulated system that balances oxygen delivery with NO-mediated vascular tone, a balance that is severely disrupted in pathological states such as sickle cell disease [18-20].

Pathophysiological Consequences in Sickle Cell Disease

In sickle cell disease (SCD), the normally balanced interaction between hemoglobin and nitric oxide (NO) becomes pathologically disrupted, primarily due to chronic intravascular hemolysis. The recurrent breakdown of sickled red blood cells releases large quantities of free hemoglobin into the plasma, where it reacts rapidly with NO. This unregulated scavenging of NO leads to a severe depletion of its bioavailability, disrupting vascular homeostasis and initiating a cascade of harmful vascular events. The loss of NO-mediated vasodilation results in persistent vasoconstriction, heightened vascular resistance, and impaired blood flow—all of which contribute to the frequency and severity of vaso-occlusive crises [21-22]. Beyond vasoconstriction, NO deficiency has profound effects on endothelial function. The endothelium becomes activated and expresses increased levels of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and selectins. This pro-inflammatory state promotes adhesion of sickled erythrocytes, leukocytes, and platelets to the vascular wall, further narrowing the vessel lumen and obstructing blood flow. These interactions play a pivotal role in the pathogenesis of vaso-occlusion and are closely linked to ischemia-reperfusion injury, tissue hypoxia, and pain episodes characteristic of SCD [23-24].

In addition to hemoglobin-mediated NO depletion, hemolysis in SCD also results in the release of erythrocyte-derived enzymes such as arginase. Arginase competes with eNOS for their common substrate, L-arginine, thereby limiting NO synthesis even further. The combined effect of substrate depletion and NO scavenging establishes a vicious cycle of oxidative stress, endothelial injury, and inflammation. Reactive oxygen species (ROS) generated during ischemia-reperfusion and from free heme exacerbate the oxidative burden, inactivating NO and amplifying endothelial dysfunction [25-26]. These pathophysiological changes contribute to the development of chronic complications in SCD, such as pulmonary hypertension, leg ulcers, stroke, and renal dysfunction. Pulmonary hypertension, in particular, is strongly associated with hemolysis-driven NO depletion and is a predictor of increased mortality in SCD patients. The inability of the pulmonary vasculature to properly dilate in response to hypoxia is directly linked to diminish NO signaling and increased oxidative stress. Moreover, recurrent endothelial activation and injury lead to vascular remodeling and contribute to the chronic progression of end-organ damage [26-27]. The systemic consequences of NO dysregulation extend beyond the vasculature. Impaired NO signaling can affect immune function, as NO also regulates the behavior of immune cells such as macrophages and neutrophils. In SCD, this can result in heightened inflammatory responses and increased susceptibility to infections and auto-inflammatory conditions. Additionally, chronic NO deficiency may impair tissue oxygenation and promote metabolic dysregulation, contributing to the overall disease burden [28-29].

Therapeutic Implications and Interventions

Given the central role of nitric oxide (NO) depletion in the pathophysiology of sickle cell disease (SCD), therapeutic strategies aimed at restoring NO bioavailability or mitigating its loss have garnered substantial research interest. One such approach involves the use of L-arginine supplementation, the primary substrate for nitric oxide synthase (NOS). Clinical trials have demonstrated that oral or intravenous L-arginine can enhance NO production, reduce pulmonary artery pressures, and improve pain control during vaso-occlusive crises. However, the efficacy of this therapy is limited by the concurrent increase in plasma arginase activity, which competes with NOS for L-arginine and curtails NO synthesis [30-31]. Inhaled nitric oxide (iNO) therapy is another targeted intervention designed to circumvent systemic NO scavenging by delivering NO directly to the pulmonary vasculature. This approach has shown transient improvement in oxygenation and vasodilation, particularly in acute chest syndrome. However, clinical trials evaluating the sustained benefits of iNO in reducing vaso-occlusive episodes have yielded mixed results. The transient nature of the response and logistical challenges of continuous inhalation therapy limit its broader applicability [32-33].

A novel class of therapeutic agents includes phosphodiesterase-5 (PDE5) inhibitors, such as sildenafil, which work downstream of NO signaling by preventing the breakdown of cyclic guanosine monophosphate (cGMP). By prolonging the vasodilatory effects of NO, PDE5 inhibitors may improve endothelial function and pulmonary hemodynamics. Although early studies in SCD patients suggested benefits in pulmonary hypertension, later trials revealed an increased risk of pain episodes, raising concerns about their safety profile and prompting a need for careful patient selection [34-35]. Efforts to neutralize free hemoglobin and reduce its NO-scavenging capacity have led to the exploration of hemoglobin-binding agents such as haptoglobin and hemopexin. These plasma proteins bind free hemoglobin and heme, respectively, facilitating their clearance and preventing oxidative and inflammatory sequelae. Recombinant forms of these scavengers are under investigation as potential therapies to protect the vasculature and maintain NO signaling. Another emerging strategy involves arginase inhibition, aimed at preserving L-arginine availability and enhancing endogenous NO synthesis [36]. Beyond pharmacologic interventions, hydroxyurea therapy, the current standard of care in SCD, exerts indirect benefits on NO bioavailability. Hydroxyurea increases fetal hemoglobin levels, reduces hemolysis, and has been shown to elevate NO metabolites through mechanisms involving eNOS activation and reduced oxidative stress. Its multifaceted effects contribute to improved endothelial function and decreased frequency of vaso-occlusive events, highlighting the importance of integrated therapeutic approaches [37].

Conclusion

The intricate interplay between hemoglobin and nitric oxide is fundamental to vascular homeostasis, yet in sickle cell disease (SCD), this relationship becomes pathologically altered due to chronic hemolysis and the subsequent release of free hemoglobin into the circulation. This leads to significant nitric oxide depletion, resulting in vasoconstriction, endothelial dysfunction, and a cascade of inflammatory and thrombotic events that contribute to both the acute and chronic complications of the disease. These mechanistic insights have highlighted nitric oxide dysregulation as a central contributor to SCD morbidity and mortality. Although some of these strategies have shown promise, others have been limited by adverse effects, inadequate efficacy, or logistical constraints. Nevertheless, the continued refinement of these therapies, alongside emerging biologics targeting NO scavenging and oxidative stress, offers hope for more effective disease-modifying interventions.

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