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Nitric Oxide and Arginine Dysregulation: A Novel Pathway to Pulmonary Hypertension in Hemolytic Disorders

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Abstract

Secondary pulmonary hypertension (PH) is emerging as one of the leading causes of mortality and morbidity in patients with hemolytic anemias such as sickle cell disease (SCD) and thalassemia. Impaired nitric oxide (NO) bioavailability represents the central feature of endothelial dysfunction, and is a major factor in the pathophysiology of PH. Inactivation of NO correlates with hemolytic rate and is associated with the erythrocyte release of cell-free hemoglobin, which consumes NO directly, and the simultaneous release of the arginine-metabolizing enzyme arginase, which limits bioavailability of the NO synthase substrate arginine during the process of intravascular hemolysis. Rapid consumption of NO is accelerated by oxygen radicals that exists in both SCD and thalassemia. A dysregulation of arginine metabolism contributes to endothelial dysfunction and PH in SCD, and is strongly associated with prospective patient mortality. The central mechanism responsible for this metabolic disorder is enhanced arginine turnover, occurring secondary to enhanced plasma arginase activity. This is consistent with a growing appreciation of the role of excessive arginase activity in human diseases, including asthma and pulmonary arterial hypertension. New treatments aimed at improving arginine and NO bioavailability through arginase inhibition, suppression of hemolytic rate, oral arginine supplementation, or use of NO donors represent potential therapeutic strategies for this common pulmonary complication of hemolytic disorders.

Keywords

Pulmonary hypertension; sickle cell disease; thalassemia; hemoglobinopathies; arginine; nitric oxide; arginase; hemolysis

Introduction

Sickle cell disease (SCD) is an inherited hemoglobin disorder that affects more than 70,000 primarily African Americans in the US, and millions more worldwide. The clinical phenotype varies widely, and is characterized by anemia, severe pain, and potentially life-threatening complications such as bacterial sepsis, splenic sequestration, acute chest syndrome, stroke and chronic organ damage. These and other manifestations result from chronic hemolysis and intermittent episodes of vascular occlusion that cause tissue injury and organ dysfunction [1, 2]. Genetically, SCD is caused by an amino acid substitution of

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valine for glutamic acid in the sixth position of the beta subunits of hemoglobin. This structural change results in intracellular polymerization under hypoxic conditions that injures and ultimately distorts the erythrocyte membrane, causing hemolytic anemia. Intracellular hemoglobin S polymerization also creates a rigid “sickled” red cell that blocks the microcirculation and gives rise to the vaso-occlusive nature of the disease [1].

Although polymerization is the primary event in its pathophysiology, SCD also affects vascular function [3]. In SCD, increased expression of adhesion molecules on erythrocytes and endothelial cells occurs, which causes young sickle red cells to adhere to the vascular endothelial cells. Interactions with leukocytes, increased levels of circulating inflammatory cytokines, reduced blood flow from increased viscosity and vascoconstriction, hemostatic activation, and endothelial damage are all thought to contribute to obstruction of the microvasculature by sickled erythrocytes [4, 5]. Ultimately these red cell changes initiate a cascade of events that results in episodic vaso-occlusion and subsequent ischemia-reperfusion injury, leading to clinical sequelae.

The thalassemia syndromes are a heterogeneous group of inherited hemoglobin disorders resulting from unbalanced production of the alpha and beta globin subunits of the hemoglobin tetramer. Thalassemia is most common in individuals whose ancestors originated from the Mediterranean region, Africa, southern China, Southeast Asia, and India. An estimated 900,000 births of clinically significant thalassemia disorders are expected in the next 20 years [6]. The clinical spectrum is a consequence of chronic hemolytic anemia and imbalanced globin chain accumulation [7]. The two clinically significant categories of beta-thalassemia have been designated thalassemia major and thalassemia intermedia. Thalassemia major is characterized by severe anemia starting during the first year of life and requiring life-long transfusion therapy for survival, while thalassemia intermedia has a later clinical onset with a milder anemia, does not typically require chronic transfusions, and offers a longer life expectancy [8].

Intravascular or intramedullary hemolysis, chronic anemia, and a high frequency of pulmonary hypertension (PH) are common features of both SCD and thalassemia. There is growing support for a new model of hemolysis-associated endothelial dysfunction and PH [9–16] that has important implications for these specific hemoglobinopathies. Hemolytic rate is also associated with a growing list of clinical complications of SCD, including multi-organ failure [17], priapism [13, 18], leg ulcers [13, 18, 19], and stroke [20], in addition to PH and mortality and constitute what is now considered the hemolytic subphenotypes of SCD [16]. Rapid consumption of NO by cell-free hemoglobin [21] coupled with the simultaneous release of erythrocyte arginase during hemolysis [22] is a fundamental aspect of this model in PH, and is the focus of this review.

Pulmonary Hypertension

PH is a vascular disorder of the lung in which the pulmonary artery pressure rises above normal levels, compromises oxygenation and right-heart function, and can ultimately become life-threatening [23]. PH is defined as a mean pulmonary artery pressure of ≥ 25 mmHg at rest or ≥ 30 mmHg during exercise, and can result from a wide range of conditions [24]. PH is a poorly understood, complex syndrome with many clinical phenotypes. The initial injury leading to pulmonary artery hypertension in different disease states may vary; however, they share a common pathway of vascular remodeling that results in a similar clinical and histopathologic condition. Histopathological findings of autopsy studies in both SCD and thalassemia include plexiform and concentric medial hyperplastic pulmonary vascular lesions, and in situ pulmonary artery thrombosis [25–30], which are common to all forms of PH. PH in SCD and thalassemia is associated with vasoconstriction, vascular

smooth muscle proliferation, and irregular endothelium in pulmonary arteries with associated thrombosis. These conditions all contribute to luminal narrowing, and eventual right ventricular failure [31] “Fig (1)”.

The responsiveness of PH to therapy will likely depend in large part upon the severity of vascular remodeling, hence early identification and treatment of PH before irreversible changes have occurred is crucial. Recent investigations suggest that endothelial dysfunction is key to the pathogenesis of pulmonary artery hypertension [32]. Because impaired nitric oxide (NO) bioavailability is a central feature of endothelial dysfunction [33], NO bioavailability represents an important target for therapy.

NO is one of the most potent vasodilators known [34] and is essential to vascular homeostasis. It plays an important role in the maintenance of vasomotor tone, limits platelet aggregation and ischemia-reperfusion injury, modulates endothelial proliferation, and possesses anti-inflammatory properties. Arginine is the precursor to NO, which is catalyzed by a family of enzymes, the NO synthases. NO causes vasodilation by activating soluble guanylate cyclase to produce the intracellular messenger, cyclic guanylate monophosphate (cGMP) [35]. Increased consumption and decreased production of both NO and arginine appear to contribute to the complications associated with PH.

Pulmonary Hypertension in Sickle Cell Disease

Pulmonary complications, which are common in SCD [36], compromise oxygenation and contribute to a vicious cycle of erythrocyte sickling. PH occurs frequently in both adults [9, 22, 37–40] and children [41, 42] with SCD. In the adult SCD population, PH occurs in >30% of patients [9], and it has been identified as a common cause of death on autopsy [26, 27, 43–45]. A Doppler echocardiogram measured tricuspid regurgitant jet velocity (TRV) of 2.5 m/sec or greater suggesting PH is currently the strongest predictor for early death in SCD, with approximately 10-fold increased risk of early mortality [9, 46, 47], and a 40% mortality risk within 3 years of diagnosis [9]. A TRV ≥ 2.5 m/sec is 2 standard deviations greater than normal.Ten percent of adult patients with SCD have a TRV ≥ 3 m/sec and of these most have mean pulmonary artery pressures greater than 25 mm Hg on right heart catheterization [48].

It remains to be determined whether this association reflects a causal relationship between PH per se and mortality, or whether PH represents a surrogate for disease severity. It has been speculated that the high risk of sudden death associated with SCD may be linked to PH. Surprisingly, despite a high risk of mortality associated with PH in SCD, it is estimated that only 10 percent of patients are monitored for pulmonary disease according to the preliminary recommendations of investigators at the National Institute of Health [40]. Even when there is monitoring, the diagnosis is often unrecognized, even with Doppler echocardiography evidence of PH [49]. This clinical misinterpretation is likely related to pulmonary pressures provoking symptoms at lower values in patients with SCD, compared to levels typically associated with PH in non-hemolytic conditions. In addition, acute elevations in pulmonary pressures occur during cardiopulmonary stressors such as pain crisis, exercise [50], and acute chest syndrome [51] and may be contributing to morbidity and mortality. Thus, universal echo screening of adult patients with SCD to identify a high-risk group who, despite the apparently mild elevation of pulmonary artery pressures [52], may be responsive to intervention has been recommended [9].

Because patients with SCD and PH are frequently asymptomatic with mildly elevated TRV [9], cases of PH are often identified only late in the course of the disease, after symptoms have developed that arouse clinical suspicion. Elevated TRV is also a common finding in children. Early studies demonstrated a prevalence similar to adults [31, 41, 42, 49, 53],

although the long-term implications of this finding in the younger patients are unknown. The average age of pediatric patients with SCD with PH receiving care at the Northern California Comprehensive Sickle Cell Center is 12.7 years, but children as young as 7 years of age exhibited echocardiogram abnormalities suggestive of PH [49]. Therefore, screening recommendations should target children as well as adults.

In one study at Children's Hospital & Research Center Oakland, children with PH had a different clinical profile of complications than adults with PH [49] (Table 1), indicating that other correlates in children may be contributing to the cause of their PH. For children, a history of sepsis, acute chest syndrome (ACS), or asthma was associated with PH, possibly reflecting distinct aspects of endothelial dysfunction. These factors are quite distinct from the high-risk profile of end-organ damage and chronic transfusion in adults associated with PH [49].

Asthma [54–57], sepsis [58–60] and ACS [61] are all conditions associated with arginine dysregulation and low NO bioavailability, a shared mechanism with hemolysis-associated PH [22, 62, 63]. Sepsis remained independently associated with PH in logistic regression analysis of these 3 significant univariate variables, with the history of asthma approaching significance [49]. Recently, sepsis has also been identified as a significant risk factor for death within five years in a neural network exploration of severity of illness in SCD [64]. It is interesting to note that in this population, a history of ACS was a risk factor for PH in children, while it was protective in adults [49], possibly reflecting the consequences of therapy intensification, including hydroxyurea use and chronic transfusion that often occurs in patients with a history of chest syndrome at our institution. A better understanding of these differing phenotypes may provide insight into mechanisms contributing to the development of PH.

Although the pathogenesis of PH in SCD is multifactorial, it is strongly linked to the intensity of hemolysis [9, 13, 16]. Increased platelet activation, implicated as another important mechanism in PH [65], likely contributes to the hypercoagulable state commonly found in patients with hemoglobinopathies [66–68], and correlates strongly with both PH severity and biomarkers of hemolysis in SCD [69]. We have found that soluble VCAM-1 is a marker of endothelial dysfunction linked to hemolytic rate [17], and that lactate dehydrogenase levels reflect hemolysis-associated endothelial dysfunction and severity of PH [13]. Plasma arginine:ornithine ratio, a reflection of arginase activity [22, 63], and brain natriuretic factor [70] are additional biomarkers of PH associated with mortality in SCD that could provide clinicians with important prognostic and diagnostic information [22, 70]. It is likely that biomarkers of hemolytic rate, either independently or in combination, may help identify a high risk sub-population in need for more aggressive interventions [9].

Pulmonary Hypertension in Thalassemia

A growing body of data suggests that thalassemia has many biologic and epidemiologic factors in common with SCD, including chronic hypoxia, long term effect of splenectomy, red cell membrane pathology [71–75], coagulation abnormalities [67, 68, 74], platelet activation [67, 68, 74], oxidative stress [76], iron overload [77–82] and chronic hemolysis [40].

Earlier studies in both thalassemia intermediate and major demonstrate that adults frequently have undetected PH, with a prevalence of 60–75% reported [79, 83–86], and a growing body of literature suggests that asymptomatic PH is a leading factor in heart failure and death in thalassemia [83]. In a study of thalassemia major, pulmonary systolic pressure above 30 mm/Hg was found in all patients over 22 years [84]. Recent PH studies in more uniformly treated thalassemia major and intermedia patients have shown a lower frequency of

increased pulmonary pressure in chronically transfused thalassemia major patients [86], while in thalassemia intermedia patients, increased pulmonary pressure was more frequently detected. In one study 60% of thalassemia intermedia patients had a pulmonary systolic pressure greater than 30 mm/Hg with preserved left ventricular systolic function [83] and another study showed a pulmonary systolic pressure greater than 35 mm/Hg in 23% of thalassemia patients [86] .

Analogous to SCD, hemolysis-associated PH is emerging as an important entity in thalassemia. [10, 62, 87]. Red cell destruction, elevated free plasma hemoglobin, anemia, and abnormal NO metabolism exist in both non-transfused and transfused patients [88]. A study of 202 TM patients concluded that strict compliance with chronic transfusion and chelation therapy to prevent iron overload reduces the occurrence of heart failure, and prevents PH [79, 86, 89]. Although more aggressive transfusion programs may provide greater protection from the development of PH, the occurrence of intramedullary hemolysis, platelet activation, iron deposits and a resultant vasculopathy may still be able to induce PH which likely progresses at a slower rate. Nevertheless, these findings strongly suggest a beneficial effect of regular transfusion in either prevention or slowing down the progression of PH in thalassemia [90].

Nitric Oxide Scavenging in Sickle Cell Disease

NO is a potent vasodilator [34], with properties that can impact many aspects of SCD: decreasing platelet activation [91, 92] and adhesion receptor expression on the vascular endothelium, decreasing vascular smooth muscle proliferation [34, 35, 93–95], limiting ischemia-reperfusion injury [96], modulating endothelial proliferation [3], and regulating inflammation [97]. Given the crucial role of NO depletion in endothelial dysfunction and PH due to other etiologies [98, 99], it is not surprising that NO depletion is associated with PH in SCD.

NO depletion has not been well studied in thalassemia [100], however given the overlap of hemolysis and oxidative stress in these hemoglobinopathies, alterations in NO bioavailability is inevitable. NO participates in the compensatory response to chronic vascular injury that occurs in SCD [61, 101, 102]. Although NO synthase expression and activity is increased [101], SCD is characterized by a state of NO resistance and increased NO inactivation [21, 102, 103]. Impaired NO bioavailability in this disorder is demonstrated by the blunted response to endothelium-dependent vasodilators in sickle cell mouse models [104, 105], as well as by a reduced flow-mediated vasodilation in human patients with SCD [102, 106, 107]. NO metabolites are elevated in patients with SCD at steady-state compared to normal controls [108], while peripheral vascular resistance [109] and resting blood pressures are low [110, 111]. Adding to this paradox, a “relative hypertension” occurs in SCD as blood pressures, while lower than in normal subjects, are higher in some patients [112, 113]. Mortality in SCD is associated with relative systolic hypertension in both male patients [113] and in patients with PH [9, 114].

Under conditions of increased hemolysis, inflammation and/or oxidative stress, the compensatory upregulation of NO likely is inadequate. Indeed, levels of NO metabolites are low during vaso-occlusive crisis [61, 115] and ACS [61], varying inversely with the degree of pain [116]. The rate of NO inactivation correlates with the hemolytic rate, and is associated with the release of cell-free hemoglobin [21]. Superoxide, already increased in SCD [117] and augmented further by circulating xanthine oxidase [104], consumes NO to form the potent oxidant, peroxynitrite [103, 118], further limiting NO bioavailability and possibly inducing cellular injury. This, in turn, could lead to increased arginine metabolism

in a compensatory attempt to produce more NO, thereby depleting arginine stores further during periods of heightened oxidative stress or hemolysis.

Finally, NO bioavailability is limited by the hemolytic process itself. As hemoglobin is liberated into plasma during hemolysis, it reacts with and destroys NO, resulting in abnormally high NO consumption and the formation of reactive oxygen species [10, 21]. Consequently, smooth muscle guanylate cyclase is not activated and vasodilation is inhibited. Reduced NO bioavailability can also cause further impairment in vascular endothelial function via transcription depression of adhesion molecules, including VCAM-1 and E-selectin, and vasoconstrictor/growth factors such as endothelin-1 [10]. The simultaneous release of erythrocyte arginase during hemolysis [22] will limit the availability of arginine to the NO synthases, further enhancing the deficiency of NO.

Novel Role of Arginase in Pulmonary Disease

Although NO has long been implicated in endothelial dysfunction, the role of elevated arginase activity in the pathogenesis of PH and other pulmonary conditions has only been recently discovered [22, 55, 62, 63, 119–123]. Arginase is an essential enzyme in the urea cycle, responsible for the conversion of arginine to ornithine and urea [124]. Found predominantly in liver and kidneys [125], arginase is also found in the red blood cells of humans and other primates [126, 127], making it an intriguing enzyme to study in hemolytic disorders. Plasma arginase activity is elevated in SCD as a consequence of inflammation, liver dysfunction and, most significantly, by the release of erythrocyte arginase during intravascular hemolysis, which has been demonstrated by the strong correlation between plasma arginase leves and cell-free hemoglobin levels and other markers of increased hemolytic rate “Fig (2)” [22].

Erythrocyte arginase release during hemolysis will alter normal arginine metabolism and is associated with the development of pulmonary complications [22]. Once released into circulation, arginase will convert arginine to ornithine, which is the precursor to proline. Proline levels are elevated in thalassemia [62] and SCD [128, 129]. Proline is an amino acid involved in collagen formation [130], lung fibrosis, airway remodeling and vascular smooth muscle proliferation [22, 121, 131], all of which are common features of pulmonary dysfunction in thalassemia [81, 132] and SCD [36, 49]. A low arginine:ornithine ratio is associated with increase mortality in SCD [22], and severity of PH of various etiologies [122]. By creating a shift towards ornithine metabolism, arginase triggers a proliferative pathway that may contribute to the clinical scenario of PH, asthma and lung fibrosis. Analogous to its proposed roles in asthma [133, 134], elevated proline levels in thalassemia [62] and SCD [128, 129] may play a role in this pulmonary pathology. Like PH, asthma is another condition that occurs more frequently in SCD than the general population [135–138] that is associated with elevated arginase activity [55, 133, 134, 139] and an acute arginine [55] and NO deficiency [54]. Although asthma is often unrecognized by clinicians, it is treatable co-morbidity associated with PH in children with SCD [49], that is also commonly identified in primary pulmonary hypertension [140]. Recently, abnormal findings on pulmonary function testing that typically occur in asthma were found to be associated with severity of PH and markers of hemolysis in patients with SCD [53]. Asthma is known to cause hypoxemia that is associated with stroke [141], ACS [136, 141, 142], and mortality [143] in SCD. Therefore, asthma in SCD should be aggressively treated based on published NIH guidelines for the diagnosis and management of asthma [144, 145], and early consultation with a pulmonary specialist is advisable. It remains to be determined whether airway hyperresponsiveness is a contributing factor or a result of PH, however dysregulated arginine metabolism and excess production of proline and polyamines likely contribute to

many forms of abnormal pulmonary function in SCD, with implications in thalassemia that warrant further evaluation.

Increased ornithine levels also may promote synthesis of polyamines, which are required for cell proliferation that occurs in vascular remodeling [133, 134]. Furthermore, since arginine and ornithine compete for the same transport system for cellular uptake (cationic amino acid transporter, CAT) [146, 147], a decrease in the arginine:ornithine ratio resulting from increased arginase activity [22, 63] could also impair arginine bioavailability for NO production, even when plasma arginine concentration appears sufficient . Altered arginase activity within the platelets of patients with SCD likely contributes to pathological platelet activation, and the hypercoagulopathy and vasculopathy in this disorder [148]. High thrombin generation is known to occur in both SCD [66, 149, 150] and thalassemia syndromes [68, 73], and contributes to a chronic hypercoaguable state. Thrombin itself increases arginase activity in human endothelial cells [151–153], and will stimulate vascular smooth muscle cell polyamine synthesis by increasing CAT and ornithine decarboxylase gene expression [154], thus playing a role in endothelial dysfunction. Additionally, Villagra and colleagues have recently demonstrated that increased platelet activation in SCD correlates strongly with both PH severity and biomarkers of hemolysis [69], while Ataga and colleagues found that measures of hemolytic rate correlated with indices of hypercoagulability [155]. A mechanistic model is emerging that links coagulation abnormalities to dysregulation of the arginine-NO pathway in PH that has important implications for hemolytic disorders.

Arginase activity is higher in the erythrocytes of patients with SCD compared to normal controls, and strongly correlates to plasma arginase activity [22]. Erythrocyte arginase activity is also elevated in thalassemia patients [156, 157]. In addition, arginase activity is higher in immature red blood cells and reticulocytes [127], compared to older cells. When these early cells are destroyed in the bone marrow, a high concentration of arginase will be released, contributing to arginine dysregulation. It is therefore likely that erythrocyte release of arginase during hemolysis will limit the availability of arginine to NO synthase, resulting in a deficiency of NO and dysregulation of arginine metabolism in thalassemia patients through a similar mechanism identified in SCD.

PH also develops in most hereditary and chronic hemolytic anemias in addition to SCD [10] and thalassemia [68, 79, 83–86, 88]; thus, erythrocyte arginase release during hemolysis may contribute to endothelial dysfunction and the pathogenesis of PH through impaired production of NO [9, 13, 22, 62, 121] and stimulation of vascular smooth muscle proliferation. [151–153] These observations support a novel mechanism of disease that links oxidative stress, chronic organ damage, hypercoagulopathy, and hemolytic rate to endothelial dysfunction and PH [9, 10, 13, 21, 62]. Arginase activity and alterations in arginine metabolic pathways have also recently been implicated in the pathophysiology of primary pulmonary hypertension and pulmonary artery hypertension associated with collagen vascular disease [122, 123], suggesting a common mechanism in the pathogenesis of otherwise distinct forms of PH.

Dysregulation of Arginine Metabolism in Sickle Cell Disease and Thalassemia

There is growing evidence that PH is a disease process that involves altered arginine metabolism or decreased arginine bioavailability [121]. Arginine metabolism is impaired in SCD “Fig.(3)” and contributes to endothelial dysfunction, PH and increased risk of mortality [22, 121]. Adults with SCD are arginine deficient at steady-state [61, 158–160], while children have plasma levels that are similar to normal controls [61]. An arginine deficiency

develops with age and is influenced by acute events [61]. There is an associated increase in erythrocyte arginine transport in SCD compared to normal controls that may be a compensatory reaction to low plasma levels [161]. Plasma arginine concentration decreases significantly in both adults and children during vaso-occlusive crisis and ACS, and is associated with low NO metabolite levels [61, 162, 163]. Ongoing intermittent vaso-occlusion may lead to a chronic depletion of arginine stores that are worsened by acute events. Of interest, acute events and exercise are also associated with worsening PH [50].

Low arginine bioavailability itself may contribute to increased consumption and decreased production of NO. Under conditions of low arginine or tetrahydrobiopterin (an essential NO synthase cofactor) availability [164, 165], NO synthase is uncoupled, producing reactive oxygen species in lieu of NO [166, 167], potentially further reducing NO bioavailability. An imbalance between eNO synthase-derived NO and superoxide generation has been established in SCD by Wood et al [168]. These authors were also the first to suggest that abnormal tetrahydrobiopterin function or availability may be yet another mechanism contributing to dysregulation of the arginine-NO pathway in SCD. This is a mechanism now well described in systemic hypertension [168–172] that has only recently been addressed in PH [173]. Upregulation of NO synthase would therefore enhance oxidative stress when arginine, tetrahydrobiopterin, or other NO synthase cofactors are deficient and NO synthase becomes uncoupled. Indeed, studies in transgenic sickle cell mice demonstrate that NO synthase activity is paradoxically increased [103, 174, 175] and uncoupled [15] in a disease state involving a marked decrease in NO bioavailability.

Arginine is an endogenously synthesized nonessential amino acid [176] that becomes essential under conditions involving an increased catabolic state such as sepsis, burn injury, and trauma, when the capacity of endogenous arginine synthesis is exceeded [177]. It is likely that SCD represents a catabolic state whereby the body's ability to maintain an arginine balance is disrupted. The majority of whole-body arginine synthesis in adults is performed in a metabolic collaboration by the small intestines and kidneys in what has been termed the "intestinal-renal axis" [130]. Intestine-derived citrulline is released into the circulation and taken up primarily by the kidneys for arginine synthesis [178]. Renal dysfunction is a common occurrence in SCD [9, 46], and is associated with PH in adults with this hemoglobinopathy [46, 49]. Kidney dysfunction will impair the major route for endogenous arginine biosynthesis, thereby contributing to a global reduction in arginine bioavailability, and likely contributes to the pathogenesis of PH. The presence of proteinuria in SCD suggests kidney dysfunction and may identify patients at increased risk for the development of PH, a finding that warrants screening for PH by echocardiography [46].

Low arginine bioavailability may be exacerbated further by the presence of elevated methylarginines, which are competitive inhibitors of arginine transport [179] and all NO synthase isozymes [180, 181]. Circulating methylarginine levels are elevated in several conditions of endothelial dysfunction, including SCD [182], and have also been implicated in the pathophysiology of systemic [182–189] and pulmonary hypertension [188, 189]. Elevated asymmetric dimethylarginine may also induce NO synthase uncoupling [190], thereby contributing to oxidative stress and dysregulated arginine metabolism in SCD.

As in SCD [22], dysregulated arginine metabolism also occurs in patients with thalassemia [62, 87]. The implications of elevated arginase activity in thalassemia remain to be determined, however, given the association of low arginine bioavailability in SCD with increased hemolytic rate, the severity of PH and mortality, this relationship in patients with thalassemia is of significant clinical interest.

Oxidative Stress and Pulmonary Hypertension

Chronic hemolysis may play an important role in the pathogenesis of PH in SCD and thalassemia. A growing body of evidence suggests additional mechanisms are also involved including a complex interaction of platelets, coagulation system, erythrocytes and endothelial cells along with inflammatory and vascular mediators. Oxidative stress also plays a pivotal role in the pathophysiology of PH [191–193]; the erythrocyte may be a major determinant of the global redox environment in hemolytic disorders such as SCD and thalassemia. The sickle and thalassemia erythrocyte have increased concentrations of reactive oxygen species compared with normal erythrocytes [194–197]. Given the presence of alterations in the glutathione buffering system that occurs in these hemoglobinopathies [196–204], it is likely that these erythrocytes are incapable of handling the increased oxidant burden, which might predispose them to hemolysis.

Recently, a depletion of erythrocyte glutamine concentration as well as aberrations in erythrocyte glutathione metabolism have been linked to PH in SCD [204]. Because alterations in the erythrocyte redox environment can contribute to both increased oxidative stress and hemolysis, the erythrocyte redox environment itself represents a mechanistic model bridging two critical pathways in the pathogenesis of PH [9, 10, 13, 16, 17, 22, 62, 191, 192, 205] in hemolytic disorders. Glutamine bioavailability may influence the redox potential of the sickle erythrocyte via the loss of nicotinamide adenine dinucleotide phosphate (NADPH) biosynthesis capacity.

NADPH is an essential co-factor required for glutathione recycling, and low bioavailability would negatively impact the conversion of oxidized glutathione (GSSG) to reduced glutathione, resulting in an overall increase in intracellular GSSG and potential efflux [206]. The downstream effects of reduced NADPH synthesis would be a loss in the overall erythrocyte pool of glutathione and a shift in the redox potential rendering them more susceptible to hemolysis. Clinical trials have demonstrated that altered erythrocyte NAD redox potential is improved by oral L-glutamine therapy [207, 208], suggesting that glutamine limitation is playing a role in oxidative stress. Dysfunctional NADPH biosynthesis has global implications due to its role as a critical co-factor for a number of enzymes, including the NO synthases and may contribute to NO synthase dysfunction [15] and further dysregulation of the arginine-NO pathway.

During NADPH biosynthesis, intracellular glutamine is metabolized to glutamate. The erythrocyte glutamine:glutamate ratio inversely correlates with both cell free hemoglobin and plasma arginase concentration as well as tricuspid regurgitant jet velocity in patients with SCD [204], suggesting a novel link between altered glutamine metabolism and hemolysis. The glutamine:glutamate ratio may therefore represent a novel biomarker of hemolysis and oxidative stress as well as PH. The value of erythrocyte amino acid analysis in SCD should be validated in future investigations, as it is more reflective of the total amino acid pools than plasma alone. In addition, selective derangement of erythrocytes amino acid pools has systemic consequences that are poorly understood. A better understanding of metabolic processes and oxidative stress occurring within the sickle erythrocyte may shed light on disease pathogenesis.

Further oxidative stress is caused by hemolysis itself and by the presence of iron overload and free-radical formation common in both SCD and thalassemia. Iron overload also affects PH by other mechanisms. It induces interstitial pulmonary fibrosis as well as left and right cardiac hemosiderosis, which subsequently results in cardiac dysfunction and affects pulmonary vascular resistance [81]. Ferriten level, a reflection of iron overload and inadequate chelation, is an independent predictor of mortality in patients with SCD [70].

Transgenic mouse models of SCD exhibit evidence of ischemia-reperfusion injury in hypoxia suggesting a role for the vascular endothelium in the modulation of the redox balance [209–212] possibly via its interactions with the sickle erythrocyte, while alteration in endothelial adhesion molecules have been linked to both PH and mortality in patients with SCD [17, 22].

Conclusion

PH is a complication observed in several hereditary and acquired hemolytic anemias [9–12, 213]. The release of hemoglobin from erythrocytes to the plasma rapidly consumes endothelial NO and increases the body's metabolic requirement for arginine. Plasma arginine bioavailability, however, is itself decreased due to the simultaneous release of erythrocyte arginase that results from hemolysis. Treatments aimed at reversing endothelial dysfunction and oxidative stress, re-coupling NO synthase, minimizing hemolysis, and enhancing arginine and NO bioavailability in hemolytic conditions including SCD and thalassemia may prove efficacious. Controlled clinical trials for the treatment of PH in these populations are needed to guide future management.

Though several drugs have been approved by the FDA for the treatment of PH, there are only limited studies of the efficacy or toxicities of these therapies in patients with hemoglobinopathies. There is currently no standard therapy recommended for hemolysis-associated PH. To date, only two studies have been published for the treatment of PH in SCD, and only case report in thalassemia. Arginine supplementation improved pulmonary artery pressures estimated by Doppler echocardiography by more than 15% after only 5 days of therapy [63], and treatment with sildenafil improved exercise tolerance in patients with SCD and PH [214]. Plans to investigate sildenafil, arginine, and therapies already approved for use in PH are in development or underway for both SCD and thalassemia. More aggressive screening and a comprehensive pulmonary exam that includes periodic Doppler echocardiography and pulmonary function testing may help identify patients at risk for PH, while early intervention may decrease mortality and morbidity for these patients and improve quality of life.

List of Abbreviations (in order of appearance)

PH	Pulmonary hypertension
SCD	Sickle Cell Disease
NO	Nitric Oxide
ACS	Acute Chest Syndrome
CAT	Cationic Amino Acid Transporter
NADPH	Nicotinamide Adenine Dinucleotide Phosphate;

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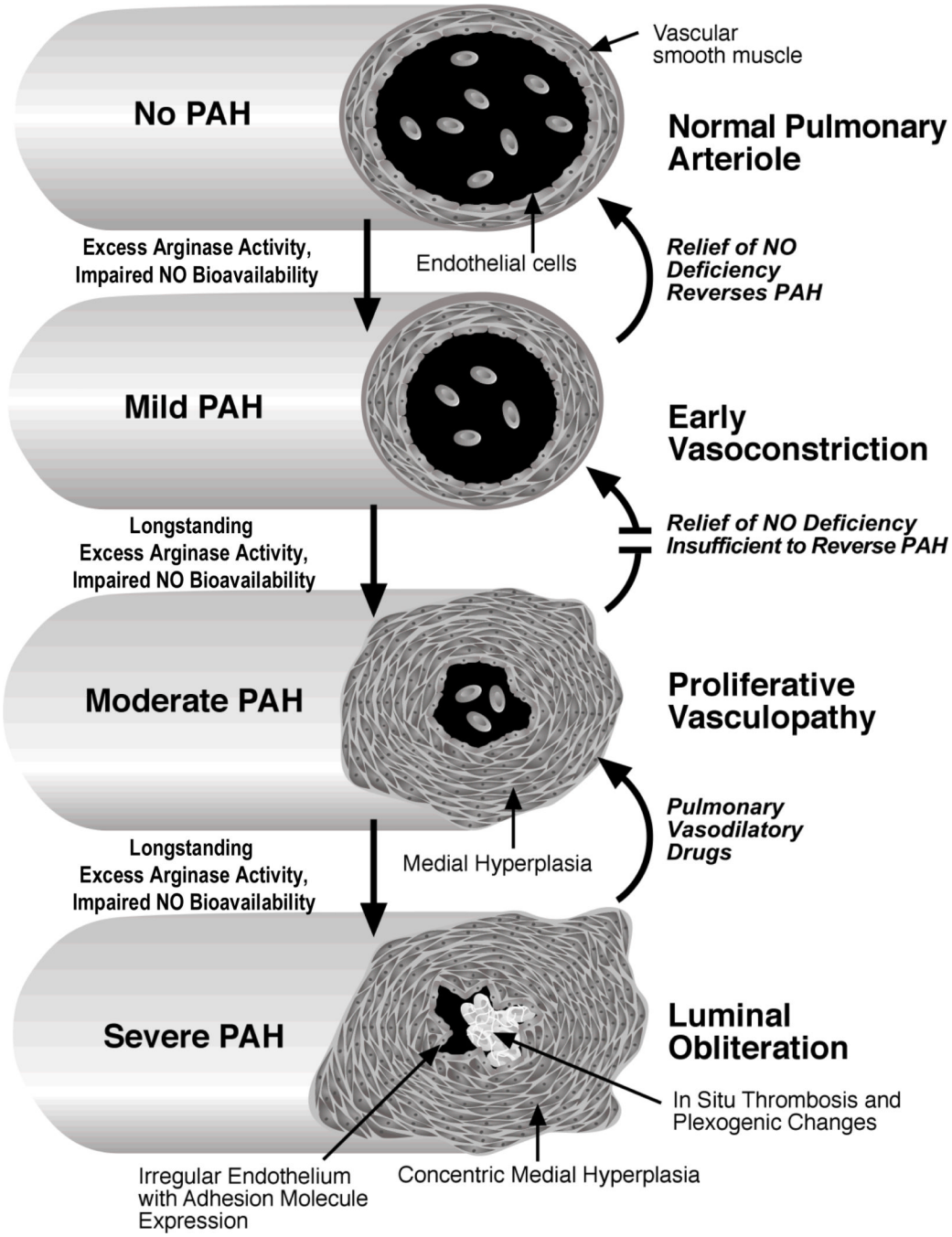

Figure 1. Progression of pulmonary hypertension in sickle cell disease and thalassemia

In this hypothetical model, impaired NO bioavailability that results from chronic hemolysis and oxidative stress triggers chronic pulmonary vasoconstriction, mildly elevating pulmonary vascular resistance and pulmonary artery pressures. Excess arginase liberated from the erythrocyte during hemolysis consumes arginine, the obligate substrate for NO production, and creates a shift towards ornithine production that contributes to collagen deposition and vascular smooth muscle proliferation. Overabundant thrombin generation contributes to a chronic hypercoagulable state, increases arginase activity, and stimulates polyamine synthesis in vascular smooth muscle cells. As this becomes more long-standing, vascular smooth muscle hyperplasia begins to create a relatively fixed lesion, compounded

in later stages by irregular, activated endothelium with expression of adhesion molecules. In situ thrombosis further occludes the vessel lumen, and results in plexogenic changes, further accelerating the progression of the pulmonary artery hypertension. (Modified Figure reproduced with permission [31]).

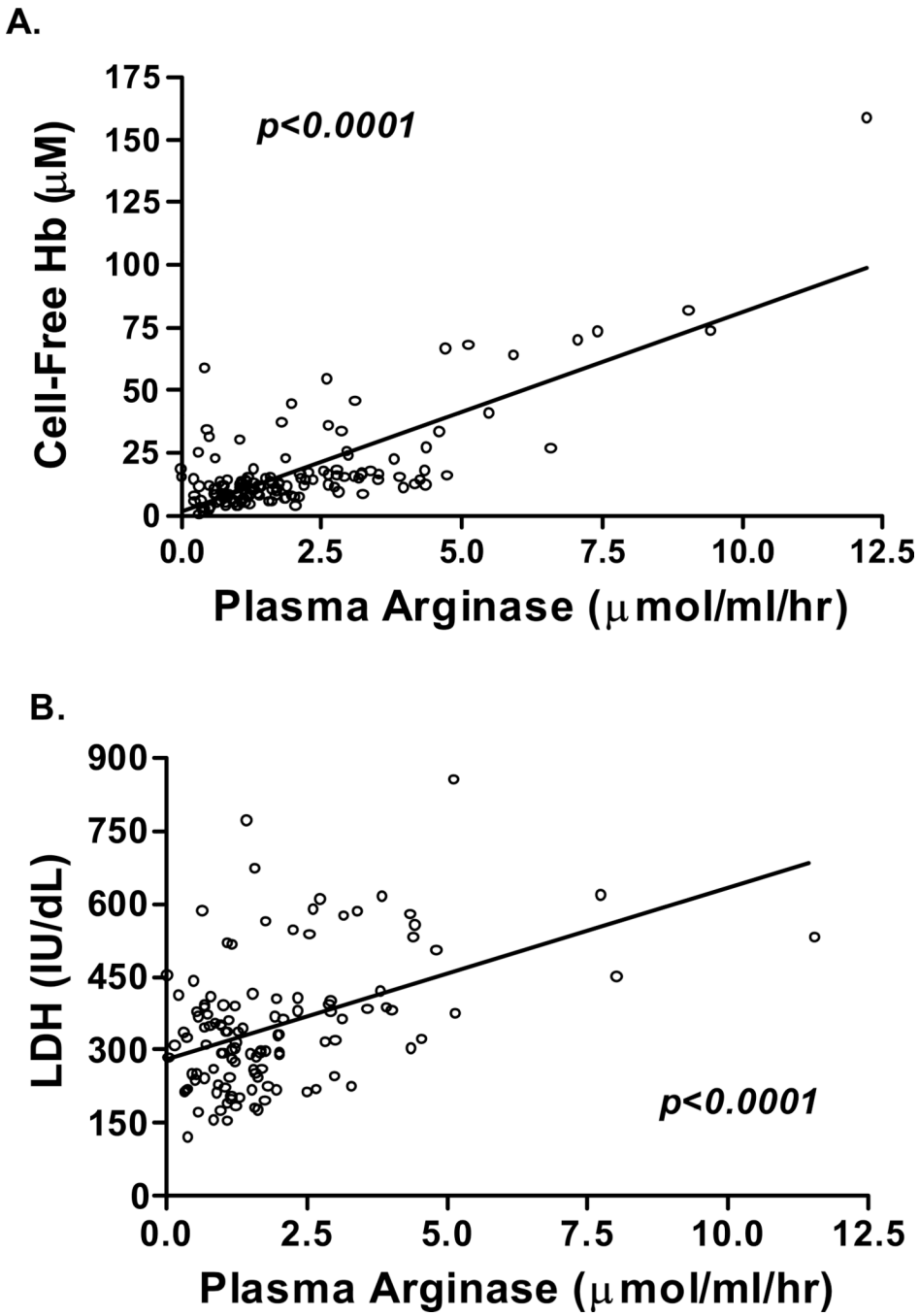

Figure 2. Association of arginase activity with hemolytic rate

Correlation of plasma arginase activity (μmol/ml/hr) to **A**.cell-free hemoglobin (*Cell-Free Hb*, n=138, $p< 0 .001$) (Reproduced from Morris et al. [22], with permission from the American Medical Association) and **B**. serum lactate dehydrogenase levels (*LDH*, n=121, $p<0.001$) in patients with sickle cell disease.

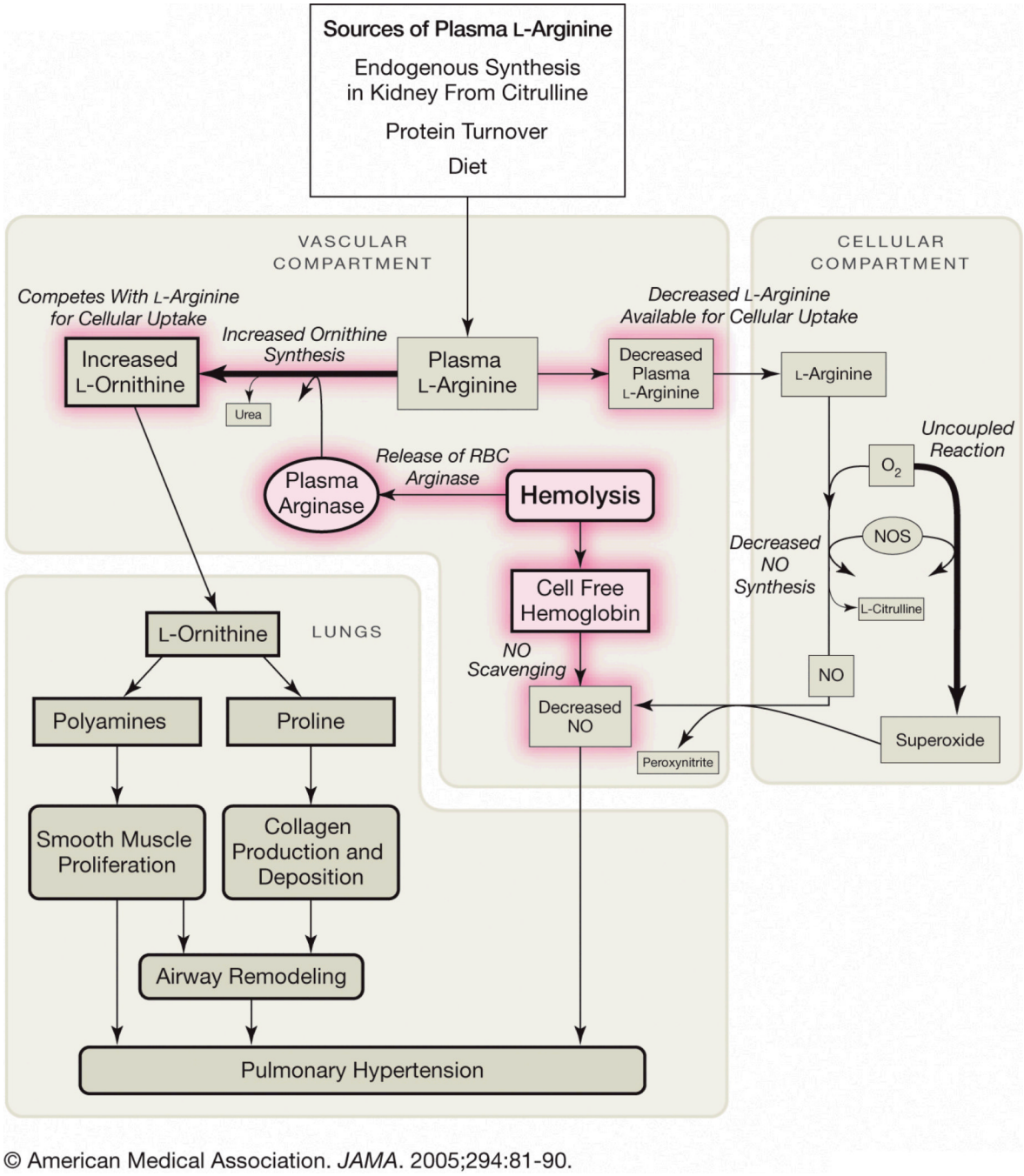

Figure 3. Altered arginine metabolism in hemolytic disorders

Arginine is synthesized endogenously from citrulline primarily via the intestinal-renal axis. Arginase and nitric oxide synthase (NOS) compete for arginine, their common substrate. In sickle cell disease (SCD), bioavailability of arginine and nitric oxide (NO) are decreased by several mechanisms linked to hemolysis, and similar mechanisms are postulated for thalassemia and other hemolytic disorders. The release of erythrocyte arginase during hemolysis increases plasma arginase levels and shifts arginine metabolism towards ornithine production, decreasing the amount available for NO production. The bioavailability of arginine is further decreased by increased ornithine levels because ornithine and arginine compete for the same transporter system for cellular uptake. Endogenous synthesis of

arginine from citrulline may be compromised by renal dysfunction, commonly associated with sickle cell disease. Despite an increase in NOS in SCD, NO bioavailability is low due to low substrate availability, NOS dysfunction, NO scavenging by cell-free hemoglobin released during hemolysis, and through reactions with free radicals such as superoxide. Superoxide is elevated in SCD due to low superoxide dismutase activity, high xanthine oxidase activity and potentially as a result of uncoupled NOS in an environment of low arginine and/or tetrahydrobiopterin concentration or potentially as a result of altered redox potential of NADPH, a critical NOS cofactor. Endothelial dysfunction resulting from NO depletion and increased levels of the downstream products of ornithine metabolism (polyamines and proline) likely contribute to the pathogenesis of lung injury, fibrosis and pulmonary hypertension. (Reproduced from Morris et al. [22], with permission from the American Medical Association).

Table 1

Clinical Variables Associated with Pulmonary Hypertension in SCD [49]

Adult Variables
Age
Renal Abnormality
Hepatitis C
Oxygen Use
Chronic transfusions
History of Acute Chest Syndrome*
Pediatric Variables
Asthma or Obstructive Disease
History of Sepsis/Bacteremia
History of Acute Chest Syndrome

* protective odds ratio in Adults only