Hemolysis-Associated Endothelial Dysfunction and Pulmonary Hypertension, an Emerging Cause of Death in the Hemoglobinopathies


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Medical advances in the management of patients with sickle cell disease (SCD), thalassemia, and other hemolytic anemias have led to significant increases in life expectancy. Improved public health with neonatal genetic screening, parental and patient education, advances in red cell transfusion medicine safety, aggressive iron chelation therapy, penicillin prophylaxis for children under 6 years of age, immunization, and hydroxyurea therapy have all likely contributed to this effect on longevity.[1-3] Now, as a generation of patients with SCD and thalassemia ages, new chronic vascular complications of these hemoglobinopathies develop.

As summarized in **Table 1**, pulmonary hypertension (PH) is emerging as one of the leading causes of morbidity and mortality in patients with hemolytic anemias, including SCD,[4-10] thalassemia (particularly thalassemia intermedia and, in cases of inadequate transfusion and chelation, thalassemia major),[11-14] paroxysmal nocturnal hemoglobinuria,[15] hereditary spherocytosis and stomatocytosis,[16,17] microangiopathic hemolytic anemias,[18] pyruvate kinase deficiency,[19] and possibly malaria.[20] Additionally, certain conditions are associated with both intravascular hemolysis and risk of PH, such as schistosomiasis,[21] and iatrogenic hemolysis from mechanical heart valves,[22] left ventricular assist devices, and cardiopulmonary bypass procedures.[23,24] Our group has received additional reports of patients with PH associated with hemolytic anemia secondary to unstable hemoglobin variants (communication, H. Franklin Bunn, and T. DeLoughery).

A common feature of both SCD and thalassemia is intravascular hemolysis and chronic anemia. Recent data suggest that chronic intravascular hemolysis is associated with a state of endothelial dysfunction characterized by reduced nitric oxide bioavailability, pro-oxidant and proinflammatory stress, and coagulopathy, leading to vasomotor instability and ultimately producing a proliferative vasculopathy, a hallmark of which is the development of PH in adulthood.[25,26] The etiology of PH in hemolytic disorders likely reflects mechanisms shared by all disorders, namely hemolysis with endothelial dysfunction and oxidant and inflammatory stress, chronic hypoxemia with activation of proliferative mediators, chronic thromboembolism and in situ thrombosis, chronic liver and renal disease, iron overload, and asplenia (**Figure 1**).

Table 1. Conditions Associated with Both Intravascular Hemolysis and Increased Risk for Pulmonary Hypertension.

Acquired hemolytic anemia
Microangiopathic hemolytic anemias
Paroxysmal nocturnal hemoglobinuria
Schistosomiasis
Mechanical heart valves
Left ventricular assist devices
Cardiopulmonary bypass devices
Malaria (?)
Hereditary hemolytic anemia
Sickle cell disease
Thalassemia
Hereditary spherocytosis
Hereditary stomatocytosis
Pyruvate kinase deficiency
Unstable hemoglobin variants

Risk Factors for Pulmonary Hypertension in Sickle Cell Disease

In the National Institutes of Health PH screening study, all markers of hemolytic anemia, including low hemoglobin and hematocrit, high lactate dehydrogenase (LDH), and high aspartate aminotransferase (released from red cells and the

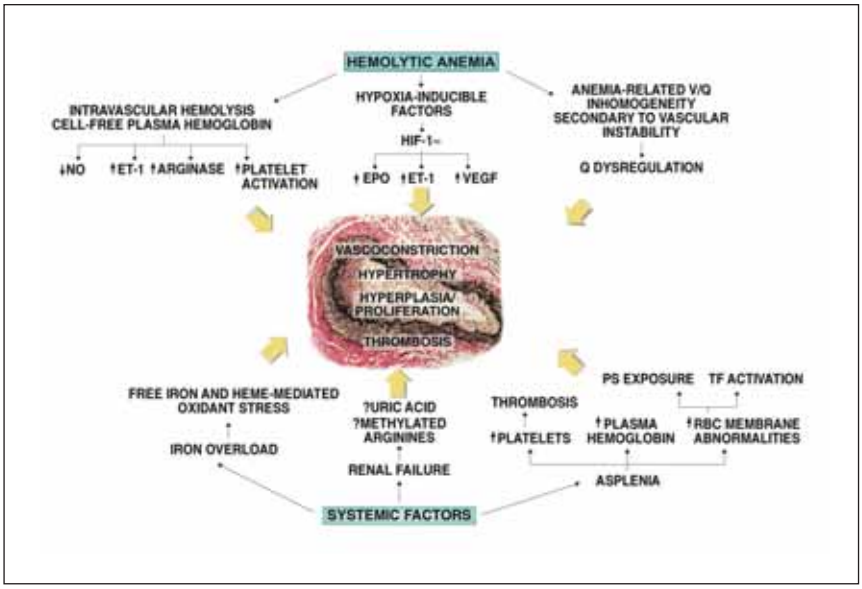

Figure 1. Pathogenesis of pulmonary hypertension in patients with hemolytic disorders. The vessel shown is an autopsy specimen from a 55-year-old man with sickle cell disease and pulmonary hypertension and demonstrates the intimal and medial pulmonary arterial proliferative vasculopathy characteristic of the disease. Mechanistic factors related to hemolytic anemia and systemic complications of sickle cell disease that may contribute to the development of this vasculopathy are shown around the vessel. NO = nitric oxide; ET-1 = endothelin 1; HIF = hypoxia inducible factor; EPO = erythropoietin; VEGF = vascular endothelial growth factor; PS = phosphatidylserine; TF = tissue factor; Q = lung perfusion. Reproduced with permission from Machado RF, Gladwin MT. Chronic sickle cell lung disease: new insights into the diagnosis, pathogenesis and treatment of pulmonary hypertension. *Br J Haematol.* 2005 May;129(4):449-464.

liver), but not alanine aminotransferase levels (released only from the liver), were associated with elevated pulmonary pressures.[9] Increasing age was also associated with a high tricuspid regurgitant jet velocity. Multiple logistic regression analysis identified a number of independent risk factors for PH: a history of renal or cardiovascular complications, increased systemic systolic blood pressure, high LDH, elevated alkaline phosphatase, and low transferrin levels (reflecting iron overload) as independent predictors of PH. In men, a history of priapism was an additional independent factor associated with PH. These associated risk factors suggest that PH is part of the systemic vasculopathy seen in some patients with SCD (systemic hypertension, renal failure, cutaneous leg ulceration and priapism) that is mechanistically linked to hemolytic rate, iron overload and cholestatic hepatic dysfunction. Interestingly, the development of PH was not associated with markers of inflammation, fetal hemoglobin levels or platelet counts, all of which are traditional risk factors for frequency of vaso-occlusive pain crisis in SCD.

Hemolysis-Associated Endothelial Dysfunction

Nitric oxide is a soluble diatomic gas molecule, much like carbon monoxide, nitrogen, and oxygen; however, since its unpaired electron is a free radical, it has unique reactivities and biological properties.[27] Nitric oxide is produced in endothelium by the endothelial nitric oxide synthase enzyme, by an oxygen-dependent conversion of L-arginine to citrulline.[28] Once produced, nitric oxide can diffuse from the endothelium to adjacent smooth muscle where it binds avidly to the heme moiety of soluble guanylate cyclase. This activates the enzyme, which in turn converts GTP to cGMP, activating cGMP-dependent protein kinases, and producing vasodilation.[29]

In addition to this vasodilation, which is tonic in nature and controls approximately 25% of our resting blood flow,[30,31] nitric oxide promotes general vascular homeostasis and health. Nitric oxide tonically down-regulates transcription of endothelial adhesion molecule genes, such as VCAM-1, ICAM-1, P-selectin, and E-selectin.[32] It inhibits platelet activation, tissue factor expression, and thrombin generation.[26] Nitric oxide modulates the expression of endothelin receptors (promoting a vasodilator effect by increases in endothelial endothelin receptor B expression) and decreases expression of endothelin 1, a potent mitogen and vasoconstrictor.[33]

In SCD and thalassemia, during intravascular hemolysis, the diffusional barriers created by the red cell membrane that limit nitric oxide reactions with hemoglobin are disrupted and the cell-free plasma hemoglobin destroys nitric oxide at a rate 1000-fold faster than intraerythrocytic hemoglobin.[26,34,35] As a result of hemolysis, hemoglobin is released into plasma where it reacts with and destroys nitric oxide, resulting in abnormally high rates of nitric oxide consumption, which produces a state of resistance to nitric oxide activity (**Figure 2**). Consequently, smooth muscle guanylyl cyclase is not activated and vasodilation is impaired. In support of this mechanism, plasma from patients with SCD contains oxyhemoglobin, which reacts with and consumes micromolar quantities of nitric oxide and inhibits forearm blood flow responses to nitric oxide donor infusions.[25] Similar effects of hemolysis on nitric oxide bioavailability and endothelial function have recently been reported in malaria.

Endothelin-1 Activation

Downstream effects of intravascular hemolysis and nitric oxide consumption include increased endothelin-1 expression, heme and free iron-mediated oxygen radical generation, platelet activation, and increased endothelial adhesion molecule expression (recently reviewed[26]). In patients with SCD, plasma endothelin-1 levels are increased in steady state and during crisis.[36-38] In vitro, sickle erythrocytes increase endothelin-1 production by cultured human endothelial cells, and endothelin receptor A antagonism decreases the vasoconstrictive effects of conditioned media from pulmonary endothelial cells exposed to sickled erythrocytes on aortic rings.[38,40] In addition, endothelin-1 activates Gardos channels in human sickle erythrocytes, an effect that may promote sickle cell dehydration and facilitate red blood cell sickling and adhesion.[41]

Intravascular hemolysis has the potential to drive a procoagulant state. Platelet activation is profoundly inhibited

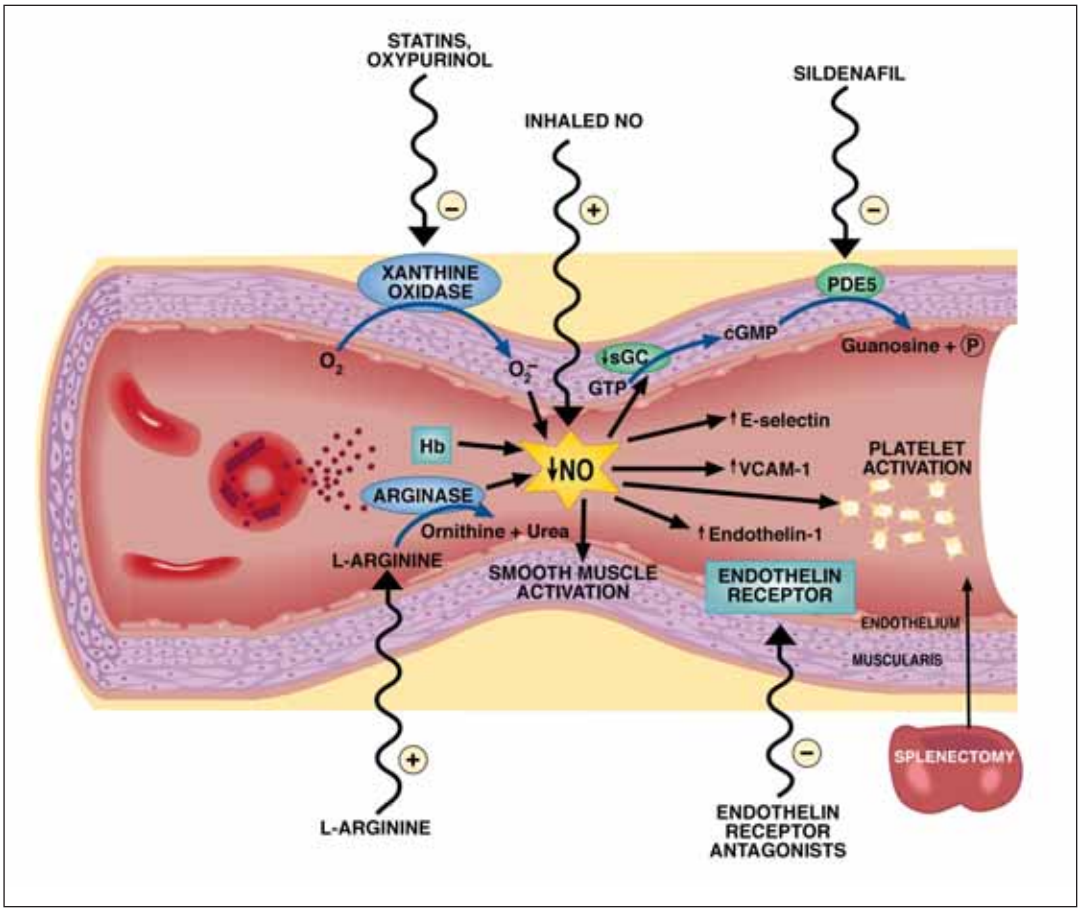

Figure 2. Pathogenesis and therapeutic targets in hemolysis-associated pulmonary hypertension and vas-culopathy. Intravascular hemolysis releases hemoglobin into plasma, which reacts with and destroys endothelial-derived nitric oxide (NO). Arginase is also released from the red cell into plasma during hemolysis and degrades arginine, further reducing NO formation from arginine. Xanthine oxidase bound to endothelium produces superoxide, which also inhibits NO. Reduced NO bioavailability promotes vasoconstriction, activation of adhesion molecules (VCAM), activation of endothelin-1, a potent vasoconstrictor, and activation of platelets and thrombosis (tissue factor). A number of therapies that target these pathways are shown on the outside of the blood vessel. Hb = hemoglobin; PDE5 = phosphodiesterase 5. Reproduced with permission from Lin EE, Gladwin MT, Machado RF. Pulmonary hypertension in patients with hemoglobinopathies: could a mechanism for dysfunction provide an avenue for novel therapeutics? *Haematologica.* 2005 Apr;90(4):441-444.

by nitric oxide and such nitric oxide-dependent inhibition may in turn be blocked by plasma hemoglobin-mediated nitric oxide scavenging.[42] Additionally, hemolytic rate (reticulocytosis) is associated with hemoglobin desaturation (ventilation/perfusion inhomogeneity) and adhesion molecule expression;[43] it is possible that such a hypoxic state can induce HIF-1-dependent factors, such as erythropoietin, VEGF, and endothelin-1. These mediators may produce a proliferative vasculopathy in the lung and other organs, such as the kidney.

Plasma Arginase

In addition to release of hemoglobin from the red cell into plasma, hemolysis releases erythrocyte arginase, which converts L-arginine, the substrate for nitric oxide synthesis, to ornithine.[44-46] Morris and colleagues found that arginase activities in the plasma of patients correlated significantly with plasma hemoglobin and LDH and were increased in the plasma and red cells of patients with SCD. Consistent with this observation, in patients with SCD, the arginine-to-ornithine ratio decreases significantly as pulmonary pressures increase and is associated with increasing mortality.[9,46]

A role for arginine supplementation as a novel nitric oxide-based therapy for SCD has been proposed.[45,47,48] Arginine therapy has been shown to decrease pulmonary pressures in patients with SCD and secondary PH [45] and has been shown to inhibit endothelin-1-mediated activation of the Gardos channel in the transgenic sickle cell mouse and thus limit erythrocyte dehydration.[49]

These pathways and novel therapeutic targets are summarized in **Figure 2**.

Asplenia

Functional or surgical asplenia may also contribute to the development of hemolysis-associated PH.[14,17,19,50, 51] It has been speculated that the loss of splenic function increases the circulation of platelet derived mediators and that senescent and abnormal erythrocytes in the circulation trigger platelet activation, promoting pulmonary microthrombosis and red cell adhesion to the endothelium.[14] Intravenous injection of hemolysate promotes the formation of platelet-rich thrombi in the pulmonary vascular bed of rabbits after ligation of the splenic artery, without any thrombus formation in the animals without splenic artery ligation.[52] A role for intensification of intravascular hemolysis by splenectomy has also been suggested by the demonstration of significantly higher plasma hemoglobin and erythrocyte-derived microvesicle levels in patients with thalassemia intermedia who have undergone splenectomy, compared with those who have not.[53] It is likely that splenic reticuloendothelial cells subserve a critical function in the removal of senescent and damaged erythrocytes and that following surgical or autosplenectomy the rate of intravascular hemolysis increases, resulting in increased plasma hemoglobin and nitric oxide scavenging, and increased circulating red cells with phosphatidylserine exposed on their membranes.

Pulmonary Fibrosis and Systemic Hypoxemia

In patients with SCD, chronic lung injury as a consequence of infection, bronchoreactive lung disease, fat embolism,

and undetected episodes of regional pulmonary hypoxia (resulting in sickling, increased vascular adhesion, and the production of vasoactive substances) may lead to chronic fibrotic pulmonary parenchymal damage, dysregulated vascular tone, regional and systemic hypoxia, vascular proliferation, and a consequent pulmonary vasculopathy. Interestingly, however, the number of episodes of acute chest syndrome (a potential cause of chronic lung disease and pulmonary fibrosis) was not associated with PH in our prospective prevalence study[9] or in a study examining the prevalence and mechanisms of PH, the Multicenter Study of Hydroxyurea therapy cohort.[54] In addition, a similar prevalence of PH in patients with thalassemia intermedia, who do not develop the acute chest syndrome, suggests that acute lung injury is not solely etiologic for PH. In our cohort, patients with PH have a higher incidence of restrictive lung disease and pulmonary fibrosis on high-resolution chest computed tomographic scanning than do age- and hemoglobin-matched patients with SCD without PH (A. Anthi, unpublished data, 2005). Furthermore, restrictive ventilatory defects and pulmonary fibrosis associated with PH have also been documented in thalassemia.[55] Taken together these data suggest that similar pathogenic proliferative mechanisms that lead to PH may underlie the genesis of pulmonary fibrosis in these patients.

Hypercoagulabilty, In-Situ Thrombosis, and Thromboembolism

A hypercoagulable state, including relatively low levels of protein C and S, elevated levels of thrombin-antithrombin complexes and D-dimers, and increased activation of tissue factor and platelets, is observed in patients with SCD in steady state.[56-61] In addition, in patients with SCD-PH, our group has found that platelet activation is prominent, and such activation is associated with high pulmonary artery pressures and with elevated markers of hemolysis, suggestive of a role for nitric oxide consumption by plasma hemoglobin in the mechanism of platelet activation (J. Villagra, unpublished data). In patients with SCD-PH, the platelets are also hypersensitive to agonist-mediated activation in vitro. This hypercoagulable state could potentially contribute to pulmonary vascular obstruction. In situ thrombosis is observed in both idiopathic pulmonary arterial hypertension and in patients with SCD.[51,62,63] Pulmonary thromboembolism is reported in patients with SCD,[63] thalassemia,[64] pyruvate kinase deficiency,[19] hereditary spherocytosis,[16] paroxysmal nocturnal hemoglobinuria,[15] and hereditary stomatocytosis.[17] However, the discrimination between in situ and embolic etiology of vascular thrombosis is not always considered or reported. Recent autopsy studies in patients with SCD suggest that much of the thrombosis is in situ, similar to what occurs in other forms of pulmonary arterial hypertension.[7] Whether thrombosis occurs secondary to the pulmonary arterial hypertension or as a primary mechanism is the subject of a current study.

Apolipoprotein Dysregulation

Using an exploratory plasma proteomics approach, our group has found evidence of dysregulated apolipoprotein expression in patients with SCD-PH (S. Yuditskaya, unpublished data). We find that SCD-PH patients tend to have lower levels of apolipoprotein A-I (apo A-I) and higher levels of apo A-II and B and serum amyloid A, resembling the apolipoprotein pattern that predisposes to atherosclerosis in the general population. We also have found that, like atherosclerosis patients, SCD patients with lower apo A-I levels have endothelial dysfunction, demonstrated by blunted vascular reactivity to doses of acetylcholine. This is fascinating, since lipid levels are significantly lower in SCD patients than in the general population, and atheroma formation is virtually absent in SCD. However, the proliferative vasculopathy of pulmonary arterial hypertension bears histopathological and biochemical similarities to that of atherosclerosis, albeit without cholesterol plaques.[7,62] This dyslipidemia pattern seen in SCD-PH emphasizes the recently described interaction of the apolipoprotein and nitric oxide pathways.[65]

Conclusions

In patients with SCD, and likely other hemolytic conditions, intravascular hemolysis produces a state of endothelial dysfunction characterized by reduced nitric oxide bioavailability and nitric oxide resistance. This leads to dysregulation of the endothelium-derived vasodilator:vasoconstrictor system, leading to acute vasoconstriction and chronic proliferative vasculopathy. Additional common complications of hemolytic anemia, such as asplenia, iron overload, and pulmonary fibrosis, likely enhance the proliferative response. These mechanisms ultimately lead to PH in the aging population, which is common in patients with all forms of hereditary hemolytic anemias, and is associated with a high risk of death in patients with SCD. New therapies targeting this vasculopathy and aimed at normalizing the vasodilator:vasoconstrictor balance are promising. PH

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