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Hemolysis-Associated Pulmonary Hypertension in Thalassemia

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Abstract

Accumulating evidence supports the existence of a condition involving hemolysis-associated pulmonary hypertension. We reported in sickle cell disease hemolysis induced release of cell-free hemoglobin, and red blood cell arginase resulting in impaired nitric oxide bioavailability, endothelial dysfunction, and pulmonary hypertension. Since thalassemia is also a condition of chronic hemolysis, these patients are at risk. Our data demonstrates that hemolysis-induced dysregulation of arginine metabolism and pulmonary hypertension also occurs in thalassemia. Erythrocyte release of arginase during hemolysis contributes to the development of pulmonary hypertension. Therapies that maximize arginine and nitric oxide bioavailability may benefit patients with thalassemia.

Introductions

PHT is a life-threatening complication observed in all hereditary hemolytic anemias [1-3] and is increasingly recognized as a major problem in patients with sickle cell disease. [1, 4-6] We have previously reported a novel disease paradigm whereby hemolysis contributes to impaired nitric oxide bioavailability, endothelial dysfunction, pulmonary hypertension and death in sickle cell disease. [7] Hemolysis results in the release of cell free hemoglobin, [8] which scavenges nitric oxide and catalyzes the formation of reactive oxygen species. Hemolysis also releases red-cell arginase, [7] an enzyme responsible for the conversion of arginine to ornithine and urea. [9] A low arginine-to-ornithine ratio correlates to severity of pulmonary hypertension and mortality in sickle cell disease. [7] Found predominantly in liver and kidneys, [10-12] arginase is also found in the red blood cells of humans and other primates. [13, 14] Arginase activity is elevated in the red blood cells of patients with sickle cell

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disease, and correlates to arginase activity found in plasma as a consequence of hemolysis. [7] Arginase activity is also elevated in red blood cells of thalassemia patients, [15, 16] and is likely related to reticulocytosis, since immature cells and reticulocytes are known to contain a high concentration of arginase. [14] It is therefore likely that erythrocyte release of arginase during hemolysis will limit the availability of arginine to nitric oxide synthase, resulting in a deficiency of nitric oxide and dyregulation of arginine metabolism in thalassemia patients through a similar mechanism identified in sickle cell disease.

Pulmonary hypertension is increasingly recognized in thalassemia as a leading factor in heart failure and death. Studies in both thalassemia intermediate and major demonstrate that adults frequently have undetected pulmonary hypertension, with a prevalence of 60-75%. [17-19] Although aggressive transfusion management has been reported to prevent the development of pulmonary hypertension in β-thalassemia major, [20] pulmonary hypertension is still common. [17-19] Thalassemia patients have many risk factors for developing pulmonary hypertension, including splenectomy, [21-23] red cell phosphatidylserine exposure, [21] coagulation abnormalities, [24, 25] and iron overload. [26] Since chronic hemolysis continues despite transfusion, these patients also remain at risk for hemolysis-associated pulmonary hypertension. We hypothesize that elevated arginase activity will contribute to dysregulated arginine metabolism in thalassemia, a potential consequence of hemolysis.

Methods and Statistical Analysis

Fourteen thalassemia patients (8 thalassemia major, 4 E-beta thalassemia, 2 hemoglobin-H alpha thalassemia) were enrolled in the study. Medical records were reviewed for transfusion history and echocardiography results. Transfusion history was considered chronic if the patient had transfusions every 4-6 weeks for longer than 5 years. All but 3 patients were on chronic transfusion therapy. Doppler echocardiography results were available on 10 patients and demonstrated 70% with pulmonary hypertension as defined by a tricuspid regurgitant jet velocity ≥ 2.5 m/s. Plasma amino acid levels and arginase activity were obtained on all patients by methods previously described. [27] Results are compared to 36 control patients without thalassemia, and are presented as mean ± standard deviation. The unpaired student t-test was used to evaluate for statistical significance. Informed consent was obtained on all patients.

Results

Dysregulated arginine metabolism occurs in patients with thalassemia. Similar to measurements in patients with sickle cell disease, [7] plasma Arg concentration trends lower in patients with thalassemia, with values ranging from normal to very low (19.5 to 122μM, median 50 μM). Ornithine levels are high, and the arginine-to-ornithine ratio is low in thalassemia patients compared to control subjects. Plasma arginase activity is significantly elevated compared to control patients (0.7 ± 0.3 vs. 0.3 ± 0.2, $p<0.001$), although a range of values is observed (0.06 - 1.17 μmol/cc/hr, median 0.83 μmol/cc/hr). Proline is also elevated, a downstream metabolite of arginase activity [28] and likely a contributor to pulmonary vascular remodeling. Citrulline, the endogenous precursor for *de novo* arginine synthesis, which occurs primarily in the kidney, [28] is also significantly elevated in thalassemia patients and may reflect impaired conversion of citrulline to arginine in patients with renal dysfunction.

Discussion

Hemolysis-associated pulmonary hypertension develops in patients with thalassemia. This is likely a consequence of erythrocyte release of arginase coupled with the liberation of cell-

free hemoglobin [8] during hemolysis that contributes to dysregulated arginine metabolism, decreased nitric oxide bioavailability and pulmonary hypertension.

Low arginine bioavailability and a shift of metabolism towards ornithine-dependent pathways are novel mechanisms that play a role in primary pulmonary hypertension and pulmonary hypertension associated with collagen vascular disease [29, 30] in addition to hemolysis-associated pulmonary hypertension, [2, 7] suggesting a greater pathophysiologic similarity in these conditions despite the diverse origins of disease. Although pulmonary hypertension is a multifactorial process, hemolysis is most likely the trigger among the hemoglobinopathies. Further evaluation is needed, however arginine dysregulation likely contributes to the high prevalence of pulmonary hypertension in thalassemia as a result of hemolysis and inflammatory pathways. Therapies that reduce hemolysis and augment arginine and nitric oxide bioavailability may benefit patients with thalassemia.

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