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Association of asymmetric dimethylarginine with sickle cell disease related pulmonary hypertension (Based on: Haematologica. 2008 Jul 18. [Epub ahead of print])

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Abstract

Reduced nitric oxide (NO) bioavailability plays a major role in the development of sickle cell disease (SCD) related pulmonary hypertension (PHT). We investigated whether asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, could play a role in SCD-related PHT.

Plasma ADMA and serum sVCAM-1 concentrations were determined in adult sickle cell patients consecutively screened for PHT with echocardiography.

Plasma ADMA concentrations were increased in HbSS/HbS β °-thalassemia patients with PHT (n=18, median 0.63 μ mol/mL, inter quartile range 0.58-0.79) as compared to those without PHT (n=28, 0.57 μ mol/mL, 0.52-0.65) (p=0.01) and significantly correlated to the haemolytic rate. Also, ADMA and sVCAM-1 were significantly correlated to the tricuspid regurgitant jet flow velocity (r_s=0.33, p=0.03 and r_s=0.49, p=0.002, respectively), and to each other (r_s=0.59, p<0.001).

ADMA may be a contributing factor to the development of SCD related PHT also linking haemolysis and endothelial activation.

Introduction

Pulmonary hypertension (PHT) occurs in approximately 30% of adult sickle cell patients and is associated with a high risk of early death. Reduced nitric oxide (NO) bioavailability secondary to chronic intra-vascular haemolysis is considered to be of primary importance in the pathogenesis of sickle cell disease (SCD) related PHT. Chronic NO shortage leads to pulmonary artery vaso-constriction, endothelial activation and vascular remodeling resulting in or contributing to an obliterative vasculopathy.

Recently we reported elevated plasma concentrations of asymmetric dimethylarginine (ADMA) in SCD, revealing another contributing factor in limiting NO bioavailability. $^{4;5}$ ADMA, as well as N^G , N^G -dimethyl-L-arginine (symmetric dimethylarginine or SDMA), derive from the irreversible post-translational methylation of arginine residues by protein arginine methyltransferases (PMRT) and are released as free amino acids upon proteolysis. ADMA (but not SDMA) competitively inhibits the NO synthase (NOS) enzyme system, thereby limiting NO production. ADMA is degraded by dimethylarginine dimethylaminohydrolases (DDAH) whereas SDMA is mainly cleared renally. Elevated plasma ADMA concentrations have been reported in several forms of PHT and have been linked to outcome in PHT as well. The aim of the current study was to investigate whether ADMA concentrations are associated with PHT in SCD.

Patients and methods

Patients

Serum and EDTA plasma samples were available to us from adult sickle cell patients consecutively screened for PHT with echocardiography as reported elsewhere. Mild and moderate-severe PHT are defined as a tricuspid regurgitant jet flow velocity (TRV) of 2.5-2.9m/s and TRV≥3m/s respectively, pulmonary-artery pressures are considered normal in patients with trace or no tricuspid regurgitation (with TRV assigned 1.3 m/s).¹ The study protocol was reviewed by a central medical ethical committee (Slotervaart Hospital) and subsequently reviewed and approved in the Academic Medical Center and Erasmus Medical Center. All patients gave written and informed consent. The study was carried out in accordance with the principles of the Declaration of Helsinki.

Laboratory determinations

Plasma concentrations of arginine, ADMA, and SDMA were measured by high-performance liquid chromatography with fluorescence detection as previously described, with modified chromatographic separation conditions. In the inter-assay coefficient of variation was <3% for all compounds. Plasma amino acids were determined by ion-exchange chromatography with ninhydrin postcolumn derivatization and colorimetric detection. Serum soluble vascular cell adhesion molecule-1 (sVCAM-1) levels were determined according to manufacturer's procedures (R&D Systems USA).

Statistics

Between group differences were tested with the Mann-Whitney U test. For correlation studies the Spearman rank correlation coefficient (r_s) was calculated. P-values <0.05 were considered statistically significant (SPSS 12.0.2, SPSS $^{\rm Inc}$, Chicago, IL).

Results

Patients

Only three of 24 HbSC/HbSβ⁺-thalassemia patients had PHT, therefore only data regarding HbSS/HbSβ°-thalassemia patients are reported (see Table 1). Our PHT patients consisted almost exclusively of patients with mild PHT (only two patients with a TRV≥3.0m/s). Use of hydroxyurea was not different between patients with and without PHT and no patients used anticoagulation or calcium antagonists, endothelin receptor blockers or sildenafil.

	without PHT	PHT	P
N	28	18	
Age (years)	33 (21-44)	28 (22-52)	0.80
Male:female	6:22	5:13	
TRV (m/s)	2.0 (1.3-2.3)	2.7 (2.6-2.8)	
sPAP* (mmHg)	21 (12-28)	34 (32-43)	
Hb (mmol/L)	5.7 (5.0-6.2)	4.9 (4.2-5.9)	0.05
HbF (%)	10.6 (6.1-18.3)	5.9 (2.2-14.1)	0.13
LDH (U/L)	369 (300-515)	575 (388-846)	0.02
GFR** (mL/min)	151 (120-195)	120 (66-172)	0.10
ADMA (µmol/L)	0.57 (0.52-0.65)	0.63 (0.58-0.79)	0.01
SDMA (µmol/L)	0.47 (0.42-0.55)	0.51 (0.47-0.83)	0.07
Arginine (µmol/L)	45 (32-56)	46 (41-62)	0.26
Ornithine (µmol/L)	56 (42-66)	56 (45-75)	0.41
Citrulline (µmol/L)	23 (16-32)	27 (20-32)	0.66
Proline (µmol/L)	208 (162-257)	209 (176-234)	0.84
Arginine/ornithine	0.84 (0.66-1.0)	0.93 (0.72-1.15)	0.45
Arginine/citrulline	1.87 (1.66-2.49)	1.98 (1.43-2.65)	0.84
Arginine/proline	0.23 (0.18-0.31)	0.25 (0.19-0.35)	0.41
sVCAM-1 (ng/mL)	1089 (801-1239)	1542 (1119-1880)	0.007

Data are presented as medians with their corresponding inter quartile range. A p-value <0.05 is considered statistically significant. *Right ventricular systolic pressure was estimated based on the modified Bernoulli equation (1) and considered to be equal to the systolic pulmonary artery pressure (sPAP) in absence of right ventricular outflow obstruction. **Cockcroft and Gault-formula (males: creatinine clearance= 1.23 x weight x (140-age) / serum creatinine, females: creatinine clearance = 1.03 x weight x (140-age) / serum creatinine).

ADMA, SDMA, amino-acid profiles and endothelial activation

Plasma ADMA concentrations were significantly increased in HbSS/HbS β °-thalassemia patients with PHT as compared to those without PHT. There were no differences in concentrations of SDMA, arginine, ornithine, citrulline and proline and their ratios between patients with and without PHT. Serum sVCAM-1 levels were significantly higher in patients with PHT (Table 1).

Both plasma ADMA and serum sVCAM-1 concentrations were significantly correlated to the TRV (r_s =0.33, p=0.03 and r_s =0.49, p=0.002, respectively), with a significant correlation between ADMA and sVCAM-1 as well (r_s =0.59, p<0.001). Statistically significant correlations of ADMA with haemoglobin concentrations (r_s =-0.47, p=0.001), HbF% (r_s =-0.50, p=0.01) and LDH levels (r_s =0.54, p<0.001) were observed. As expected, SDMA, but not ADMA, concentrations were significantly correlated to the glomerular filtration rate (GFR) (r_s =-0.66, p<0.001, r_s =-0.08, p=0.60, respectively). SDMA was also significantly correlated to ADMA concentrations, haemoglobin concentrations (r_s =-0.51, p=0.001), and LDH (r_s =0.31, p=0.04). Haemoglobin concentrations were significantly correlated to TRV (r_s =-0.30, p=0.04). There was no statistically significant correlation between age and TRV, ADMA concentrations, haemoglobin concentrations, LDH levels and sVCAM-1 levels (data not shown), whereas both GFR en SDMA were significantly correlated to age (r_s =-0.58, p<0.001, r_s =0.52, p<0.001, respectively).

Discussion

Haemolysis driven reductions in NO bioavailability as a result of both NO scavenging by cell free haemoglobin and increased arginase activity are of major importance in the pathophysiology of SCD related PHT.² Over the last years ADMA has been recognized as an important regulator of NO production by inhibiting NOS activity.⁶ and plasma ADMA concentrations are elevated in SCD.^{4;5} In this study we demonstrate an association of plasma ADMA concentrations with SCD related PHT, haemolysis and endothelial activation.

Plasma ADMA concentrations were higher in patients with PHT as opposed to patients without PHT, with a modest but statistically significant correlation between the TRV and plasma ADMA concentrations. ADMA concentrations in patients without PHT were elevated as compared to values previously reported by us in healthy race matched controls.^{4,5} Clearly, patients with the highest haemolytic rate are characterized by highest plasma ADMA and SDMA concentrations indicating that the haemolytic rate may be an important determinant of methylarginine production in SCD (likely due to the increased protein turn-over in the stress erythropoiesis). Specific factors related to the pulmonary vasculature contributing to ADMA increments in SCD could be shear stress induced PMRT activity and hypoxia induced DDAH down–regulation.¹²⁻¹⁴ Although the ADMA difference between patients with and without PHT seems modest, even small extra-cellular ADMA increments lead to significant intra-cellular NOS inhibition through preferred cellular uptake of ADMA over arginine.⁶ A recent study demonstrated a clear association of plasma ADMA concentrations ≥0.64µmol/L with strongly reduced pulmonary artery endothelial NOS expression and early death in patients with thrombo-embolic PHT.⁸

ADMA induced NOS inhibition is associated with endothelial activation and dysfunction. ^{15;16} In our study serum sVCAM-1 levels strongly correlated to plasma ADMA concentrations, indicating that ADMA induced NOS inhibition may be an important contributor to the characteristic endothelial activation of SCD. ¹⁷ As reported by others, serum sVCAM-1 levels correlated significantly to the TRV in our patients. ¹⁸ Given the strong relation of ADMA to endothelial activation, it would be interesting to hypothesize that chronic haemolysis induced ADMA elevations significantly contribute to endothelial activation and dysfunction via NOS inhibition in SCD and that patients with higher ADMA concentrations are more prone to develop a vasculopathy leading to complications such as PHT over time.

Previous studies have demonstrated an association of increased arginase activity (reflected by lower arginine to ornithine ratios) with severe, but not mild SCD related PHT.^{1;19} In accordance with the studies above, no difference in arginine/ornithine ratios between our patients with mostly mild PHT and patients without PHT could be detected, indicating similar arginase activity. The

increased plasma ADMA concentrations in mild SCD related PHT therefore could suggest a role of pathophysiological importance at an earlier stage than increased arginase activity.

In interpreting these data the relatively small number of patients needs to be taken into account and these findings need to be reproduced in a larger cohort. Also, right heart catheterization remains the gold standard diagnostic test for PHT and is recommended in sickle cell patients with moderate-severe PHT.² However, given the reported excellent correlation between pulmonary artery pressure and TRV in SCD,¹ and the fact that an elevated TRV is the result of solely left-sided heart disease in only a minority of cases,²⁰ we do not feel that the lack of right heart catheterization would significantly affect our results.

Taken together, our data identify a potential role of ADMA as a novel early contributing factor to the development of PHT in SCD. Also, ADMA induced limitation of NO production may well provide an important new mechanistic link between haemolysis and the characteristic endothelial activation of SCD.

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Chapter 9

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