Asymmetrical dimethylarginine in systemic sclerosis-related pulmonary arterial hypertension

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Introduction

SSc is a multisystem disorder characterized by extensive vascular damage and fibrosis of the skin and internal organs. Endothelial dysfunction and damage are primary events throughout the course of the disease, which result in vascular obliteration and diminished blood flow to the organs involved. SSc-related pulmonary arterial hypertension (SScPAH) is a complication of the disease, which may occur as a result of interstitial fibrosis, or of direct proliferative vascular involvement in the absence of significant parenchymal disease [1]. In SSc patients, pulmonary arterial hypertension (PAH) has become the leading cause of morbidity and mortality, with reports that up to 60% of these patients die due to PAH [2].

The free radical nitric oxide (NO) is a potent endothelium-derived vasoactive mediator and is synthesized from l-arginine by NO synthase (NOS). Endogenous guanido-substituted analogues of l-arginine that can selectively inhibit NOS have been implicated in the pathogenesis of various cardiovascular diseases [3]. Asymmetrical dimethylarginine (ADMA) is the most potent of them and represents a novel risk factor for endothelial dysfunction. High ADMA levels predispose to acute coronary events, correlate with the severity of atherosclerotic disease and predict mortality and cardiovascular events in patients with end-stage renal disease [4, 5]. Elevated ADMA levels have been reported in patients with idiopathic PAH (IPAH) and have been described as an independent predictor of mortality [6].

The aim of our study was to investigate the potential relationship between serum ADMA and pulmonary hypertension and functional capacity in patients with SSc.

Objectives.

SSc is a CTD characterized by vascular involvement, with generalized disturbance of the microcirculation, which may lead to pulmonary artery hypertension (PAH). Asymmetrical dimethylarginine (ADMA) is an endogenous nitric oxide (NO) inhibitor. Increased concentrations of plasma ADMA may also contribute to endothelial dysfunction in patients with pulmonary vascular disease. The aim of our study was to elucidate the possible relationship between serum ADMA and PAH in patients with SSc. Moreover, we sought to investigate the effect of ADMA levels on the functional capacity of these patients.

Methods.

Plasma ADMA levels were measured in 66 patients with SSc (63 females, mean age 57.8 ± 12.8 yrs, median duration of disease 9.9 yrs, 47 with lcSSc and 19 with dcSSc disease) and 30 healthy controls (29 females, mean age 58.2 ± 8.4 yrs). Systolic pulmonary artery pressure (sPAP) assessed by echocardiography, lung function tests, 6-min walk test (6MWT) and serum ADMA levels were recorded from patients.

Results.

In 24 patients, the diagnosis of PAH was established. Mean value of ADMA for SScPAH patients was 0.44 ± 0.22 μmol/l compared with 0.26 ± 0.18 μmol/l for patients without PAH and 0.25 ± 0.20 μmol/l for controls (P = 0.001). ADMA levels were inversely correlated with the 6MWT (r = −0.55, P = 0.005).

Conclusions.

In patients with SScPAH, increased ADMA serum levels and their negative association with exercise capacity suggests that the NO pathway is involved in the development of pulmonary vascular disease.

KEY WORDS: Scleroderma and related disorders, Biochemistry, Biomarkers, Ultrasonography.

Patients and methods

Study population

We prospectively studied 66 SSc patients as defined by the revised ACR criteria [7] who visited our centre for follow-up care. For comparison, 30 healthy controls (29 females, mean age 58.2 ± 8.4 yrs) matched with medical comorbidities, such as coronary artery disease and chronic heart failure, with respect to renal function, mean arterial pressure, cholesterol and triglyceride levels, were examined. SSc patients with symptomatic lower limb vascular disease were excluded. All patients were evaluated for renal, cardiac, and pulmonary involvement. Exercise capacity was assessed by 6-min walking test (6MWT). The study received ethical approval from the scientific committee of the Aristotle University of Thessaloniki and patients provided written informed consent.

In all patients, blood samples were drawn and analysed for routine laboratory parameters, including Westergren ESR, CRP and homocysteine levels. Plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) concentration was also measured by using a commercial enzyme immunoassay kit (Biomedica, Vienna, Austria).

Pulmonary function testing

Pulmonary involvement was assessed by high-sensitivity CT and lung function tests (spirometry and single-breath diffusing capacity).

Echocardiography

Complete 2D and Doppler echocardiographic examinations were performed in all patients at the same day of ADMA evaluation. We used a commercially available system (Vivid 7, GE Vingmed, Horten, Norway). From the apical 4-chamber view, RV inflow view and parasternal short axis or subcostal view, continuous-wave Doppler echocardiography was used to assess the peak tricuspid regurgitant velocity [8]. Using the simplified Bernoulli equation (ΔP = 4V²), the pressure gradient across the tricuspid valve was calculated. The right atrial pressure was estimated using...
the diameter of the inferior vena cava and the response to changes in respiration [9]. Systolic pulmonary artery pressure (sPAP) was determined by this way and PAH was defined as an sPAP value ≥40 mmHg [10].

Exercise capacity

The 6MWT was performed using a standardized protocol in accordance with guidelines in the American Thoracic Society statement 2002 [11]. Patients walked an enclosed, level corridor unassisted; length to first turn around was 30 m. All patients were told to use their own pace, but to cover as much ground as possible in 6 min. All patients underwent the 6MWT before echocardiographic evaluation and blood sample ADMA measurement.

Measurements and calculation

Concentration of ADMA was measured in serum samples by using a commercial enzyme immunoassay ELISA kit (DLD Diagnostica, Hamburg, Germany). The kit uses an immunoaffinity, highly specific and sensitive rabbit anti-ADMA antibody. The ADMA concentrations obtained and the performance of the ELISA have been found to be consistent with other widely applied methods used to quantify ADMA, such as gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry [12].

Statistical analysis

Data are mean ± s.d. or number except for NT-proBNP and duration of disease, which are expressed as median [25th–75th percentile]. Categorical data are presented as absolute values and percentages, and comparisons were tested by Fisher’s exact test. Clinical and biological characteristics in SSc patients with and without PAH were compared using the Student’s t-test or the non-parametric (Mann–Whitney) test as appropriate. Differences between the three groups (SSc patients with and without PAH and controls) were compared using one-way ANOVA with post hoc analysis. The relationship between ADMA and patient’s functional state as measured by the 6MWT was assessed by the Pearson’s correlation coefficient. A value of P < 0.05 was considered significant. The statistical software used was SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

We evaluated 66 patients (63 females, mean age 57.8 ± 12.8 yrs, median duration of disease 9.9 yrs, 47 with lcSSc and 19 with dcSSc) and 30 age-matched healthy controls. The demographic and clinical characteristics of the patients and the controls are detailed in Table 1.

In 24 patients, the diagnosis of PAH was established. Mean value of ADMA for SscPAH patients was 0.44 ± 0.22 μmol/l compared with 0.26 ± 0.18 μmol/l for patients without PAH, and to 0.25 ± 0.20 μmol/l for controls (P = 0.001, Fig. 1). In the subgroup of SscPAH patients, ADMA levels were inversely correlated with the 6MWT (r = −0.55, P = 0.005, Fig. 2). No significant correlations were found between ADMA and serum creatinine, forced vital capacity, forced expiratory volume in 1 s, total lung capacity (TLC) and diffusing capacity for carbon monoxide values. The only trend for statistical significance was observed between ADMA and NT-proBNP levels (r = 0.36, P = 0.08). Moreover, no association was found between ADMA and the presence of interstitial lung disease associated with SSc-ILD or other complications of the disease (digital ulcers, oesophageal insufficiency).

Discussion

The main finding of this study is that the plasma ADMA levels are significantly higher in SScPAH compared with those without PAH and healthy controls. We also demonstrated an inverse correlation between ADMA concentration and functional capacity measured by the distance walked during 6MWT.

Our results are in line with those of Kielstein et al. [6] who found that ADMA plasma levels are increased in patients with IPAH. To the best of our knowledge this is the first time that a rise of ADMA levels has been reported in SScPAH. Increased levels of ADMA have been reported in patients with diffuse scleroderma [13], but no correlation with PAH was mentioned.

| TABLE 1. Clinical and biological characteristics of patients with SSc |
|------------------|------------------|------------------|------------------|
|                   | All SSc patients | SSc patients without PAH | SScPAH patients |
|                   | (n = 66)         | (n = 42)           | (n = 24)         |
| Age, mean ± s.d., yrs | 57.7 ± 12.1   | 55.3 ± 13.1  | 60.1 ± 1  |
| Gender, F/M        | 63/3            | 41/1            | 22/2           |
| Duration of disease, median (25th to 75th percentile), yrs | 19 (0.2–31) | 9.7 (0.2–31) | 11 (12–25) |
| LcSSc/dcSSc        | 47/19          | 28/14          | 19/5           |
| Pulmonary fibrosis on HRCT | 30             | 19             | 11             |
| Digital ulcers     | 18              | 11             | 7              |
| Oesophagus insult  | 39              | 23             | 16             |
| FVC, mean ± s.d., % predicted | 86.8 ± 22.6 | 88 ± 25.4 | 83 ± 18 |
| FEV1, mean ± s.d., % predicted | 89.4 ± 23.9 | 89.8 ± 26.8 | 88 ± 20 |
| TLC, mean ± s.d., % predicted | 78.5 ± 18.2 | 80 ± 18 | 76 ± 17.7 |
| DLCO, mean ± s.d., % predicted | 65.4 ± 22.1 | 65.5 ± 22.1 | 62 ± 21 |
| EF, mean ± s.d., % | 65.5 ± 4.2      | 65.5 ± 4.4     | 65.4 ± 4.8    |
| SPAP, mean ± s.d., mmHg | 31.6 ± 6.8 | 26 ± 4.5 | 46.2 ± 5.1 |
| Cholesterol, mean ± s.d., mg/dl | 208.6 ± 26.2 | 209 ± 37 | 204.7 ± 36 |
| Triglyceride, mean ± s.d., mg/dl | 154.3 ± 79.9 | 140.2 ± 72.4 | 171 ± 85.6 |
| HDL, mean ± s.d., mg/dl | 63.5 ± 20.8 | 62.4 ± 15.9 | 66.3 ± 27.2 |
| LDL, mean ± s.d., mg/dl | 117 ± 33.5 | 115 ± 32 | 100 ± 35.3 |
| Creatinine, mean ± s.d., mg/dl | 0.73 ± 0.1 | 0.8 ± 0.1 | 0.73 ± 0.1 |
| ESR, mean ± s.d. | 32.5 ± 2        | 30.5 ± 21      | 34.5 ± 20     |
| Homocysteine, mean ± s.d., μmol/l | 12.3 ± 4.9 | 11.4 ± 5 | 13.6 ± 4.8 |
| CRP, mean ± s.d., mg/ml | 0.46 ± 0.1 | 0.5 ± 0.3 | 0.4 ± 0.2 |
| Prednisolone treatment (5–10 mg/day) | 38 | 23 | 15 |
| NYHA, ll/l/l/l | 24/32/10/10      | 22/19/11/10    | 5/10/9        |
| SMWT, mean ± s.d., metres | 405.4 ± 106.4 | 423 ± 98 | 383 ± 113 |
| NT-proBNP, median (25th to 75th percentile), fmol/ml | 382.7 (83.8–2254.3) | 304 (83.3–592) | 514 (116–2254.3) |
| ADMA, mean ± s.d., μmol/l | 0.35 ± 0.23 | 0.26 ± 0.18 | 0.44 ± 0.22 |

*Comparison between SSc patients without PAH and patients with SScPAH patients. HRCT: high-resolution CT; FVC: forced vital capacity; FEV1: forced expiratory volume in 1 s; DLCO: diffusing capacity for carbon monoxide; EF: ejection fraction of left ventricle; HDL: high-density lipoprotein; LDL: low-density lipoprotein; 6MWT: six-minute walking test; TLC: total lung capacity; NYHA: New York Heart Association.
NO has vasodilating effects on vessels and inhibits the thrombogenicity and the proliferation of vascular muscle cells [14]. Constitutive production of NO is important for the regulation of blood flow and the maintenance of normal vascular wall structure. Reduced endothelial NOS expression has been identified in the lung of patients with PAH [15]. It is possible that the inhibition of NOS by increased levels of ADMA, may contribute to pulmonary vasoconstriction and to the excessive growth of the tunica media observed in SScPAH.

ADMA is released from proteins that have been posttranslationally methylated and subsequently hydrolysed, and is metabolized by the enzyme dimethylarginine dimethylamino-hydrolase (DDAH), which hydrolysates ADMA to dimethylarginine and L-citrulline [16]. A recent study in transgenic mice that overexpress DDAH provided compelling evidence that the metabolism of ADMA plays an important role in regulation of NOS activity [17]. Moreover, increased levels and reduced catabolism of ADMA due to suppression of endothelial DDAH expression and function was found in both human lung tissue of IPAH patients and the tissue of monocrotaline-induced PAH in rats [18].

Previous findings suggest that NO metabolism may be impaired in pulmonary involvement in SSc patients. It has been demonstrated that exhaled NO is increased in SSC-ILD [19, 20], but that decreased levels are found when SScPAH is present [21–23]. These results may explain the potential pathogenetic role of ADMA and reinforces our hypothesis that increased ADMA concentration down-regulates the production of NOS in SScPAH patients. On the other hand, no association between ADMA levels and SSc-ILD was found in our study.

Endothelial dysfunction in SSc is considered to be among the primary events during the progression of the disease. Reduced intracellular NOS production was described in microvascular endothelial cells derived from SSc patients [24–26]. These data suggest a key change in the endothelium phenotype that may result in or predispose to the disease [27]. In our study, SSc patients without PAH had increased ADMA concentrations as compared with healthy controls. This finding may reflect the abnormal regulation of NO production due to the derangement of endothelial function.

It is widely accepted that the 6MWT should be determined in the management, evaluation and follow-up assessment of patients with SScPAH. Our results showed inverse correlation between ADMA levels and impaired functional status resulting from right heart failure in SScPAH. Additionally, we found a trend for significant positive correlation between NT-proBNP and ADMA levels. NT-proBNP levels increase in proportion to the degree of right ventricular dysfunction [28] and have been described as a marker of PAH in patients with SSc [29–31]. If plasma ADMA levels have a similarly equivalent prognostic accuracy, then our ability to monitor patients with SScPAH and adjust their therapy will be significantly improved.

The most important limitation of our study is that the patients did not undergo right heart catheterization, the gold standard for the diagnosis of PAH. Nonetheless, a 2D transthoracic echocardiography is considered an excellent, first-line, diagnostic test for these patients [32]. Moreover, due to the lack of right heart catheterization, blood samples for measurement of plasma ADMA concentrations were obtained from the antecubital vein.

In conclusion, increased ADMA serum levels and their negative association with exercise capacity suggest that the NO pathway is involved in the development of pulmonary vascular disease in scleroderma. Further investigations in prospective studies will unfold in detail the pathophysiological role of ADMA in SScPAH, and the potential use of NO/ADMA system as a therapeutic target in the future.

**Rheumatology key messages**

- NO/ADMA pathway may contribute to the endothelial dysfunction resulting in pulmonary vascular disease in SSc.
- ADMA levels are inversely correlated with functional capacity in SScPAH patients.

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