Elevated Plasma Adrenomedullin and Vascular Manifestations in Patients with Systemic Sclerosis

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ABSTRACT. ***Objective.*** Adrenomedullin (ADM), a vasodilating peptide that possesses antiinflammatory properties, may have a regulatory role in the vascular manifestations of scleroderma (systemic sclerosis, SSc). We examined associations between ADM concentrations and vascular manifestations in a cohort of patients with SSc.

Methods. Patients were examined for manifestations of severe Raynaud's phenomenon (RP), defined as digital resorption, previous iloprost infusion, and sympathectomy. Doppler echocardiography and lung function tests were performed to detect elevation in pulmonary arterial pressure (PAP; > 35 mm Hg) and interstitial lung disease (ILD). Plasma ADM was measured by radioimmunoassay.

Results. Plasma ADM was measured in 62 SSc patients and 21 healthy controls. Elevated PAP was found in 15 (24.2%) SSc patients (mean PAP 46.5 ± 11.2 mm Hg, range 37–74). ADM was not found to be related to age, sex, disease duration, or clinical subset. ADM level was significantly higher (median 13.9 pmol/l) in SSc patients with elevated PAP compared to those with lower PAP (median 7.2 pmol/l) ($p = 0.01$) and controls (median 7.9 pmol/l) ($p = 0.04$). ADM level was not different among patients who had elevated PAP with ($n = 10$) and without concomitant ILD ($n = 5$) ($p = 0.21$). SSc patients with severe RP (38.7%; median ADM 11.9 pmol/l) were found not to have different ADM levels compared to controls ($p = 0.75$). Patients who had both severe RP and elevated PAP were found to have significantly higher ADM levels (median 22.3 pmol/l) than patients who had neither manifestation (median 8.0 pmol/l) ($p = 0.006$) and those with severe RP alone (median 4.2 pmol/l) ($p = 0.006$).

Conclusion. Elevated ADM was found in SSc patients with increased PAP regardless of concomitant ILD. (First Release Oct 15 2007; J Rheumatol 2007;34:2224–9)

Key Indexing Terms:
CONNECTIVE TISSUE DISEASE
PULMONARY ARTERIAL HYPERTENSION
ENDOTHELIN-1
ADRENOMEDULLIN

Adrenomedullin (ADM) is a 52-amino acid peptide that is involved in many physiological and pathological processes, including angiogenesis, growth regulation and differentiation, vasodilation, and smooth-muscle relaxation[1]. The ADM gene is expressed in many different cell types in various tissues[1]. It is highly expressed in vascular endothelial cells and smooth-muscle cells that actively produce and secrete ADM into the circulation[2,3]. ADM is a potent vasodilator, acting directly on vascular smooth-muscle to increase intracellular cAMP and stimulation of endothelial nitric oxide release[4]. ADM also possesses antiinflammatory actions, as shown by its inhibitory effect on the production of chemoattractants from alveolar macrophages[5] and its antiapoptotic action on endothelial cell *in vitro*[6]. Elevated levels of ADM have been described in rheumatic diseases including systemic lupus erythematosus[7] and rheumatoid arthritis[8] and have been postulated to be related to inflammation in these conditions.

Systemic sclerosis (SSc) is a connective tissue disease characterized by sclerodermatous skin changes, Raynaud's phenomenon (RP), and internal organ fibrosis. The various vascular manifestations of SSc including RP, abnormal nailfold capillary changes, and pulmonary hypertension suggest endothelial dysfunction as an underlying pathogenic mechanism. Further, increased production of proinflammatory cytokines including tumor necrosis factor-α, interleukin 1ß, and interferon-γ have been described in involved tissues of SSc[9]. These cytokines have been shown to induce ADM expression through nitric oxide-dependent and independent pathways[10]. As the concentration of ADM has been shown to be elevated in SSc patients with RP and pulmonary hypertension in small case series[11,12], our study examined the association of ADM levels with severity of vascular manifestations of a larger cohort of patients with SSc.

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MATERIALS AND METHODS

Patients. Consecutive patients with SSc were recruited from a university affiliated rheumatology clinic in Hong Kong. The study was approved by the Clinical Research Ethics Committee of the Institutional Review Board of The University of Hong Kong/Hospital Authority Hong Kong West Cluster. A cross-sectional study was performed to study vascular manifestations including severity of RP and presence of elevated pulmonary arterial pressure (PAP) in these patients. Echocardiography, lung function test, and blood sampling were done on the day of clinic visit. Data on patient demographics, serological results, and medications were retrieved from medical records. SSc was designated as limited (lSSc) or diffuse (dSSc) on the basis of the extent of skin involvement according to the method described by LeRoy, *et al*[13]. Limited disease was defined as skin thickness confined to areas of the extremities below the elbows and below the knees. Diffuse disease was defined as skin thickness involving the proximal extremities or the trunk below the clavicles. Patients were examined for ischemic digital ulcer, scars, or finger pulp resorption. Patients who had had previous iloprost infusion and sympathectomy were also regarded as having severe RP. Pulmonary involvement was defined as < 80% predicted of one or more of the measures on lung function including forced vital capacity (FVC), diffusing capacity for carbon monoxide (DLCO), and/or diffusing coefficient (KLco).

Echocardiography. M-mode and cross-sectional continuous Doppler and color Doppler echocardiography (Sonos 1000, Hewlett Packard, Burlington, MA, USA) were performed by the same cardiologist. Measurements were taken with the patient lying in the left lateral position, using a phased-array 2.5-MHz transducer. The thickness and gradient across heart valves, color Doppler, and left ventricular ejection fraction were examined to exclude other heart diseases. Tricuspid regurgitant flow was identified with color Doppler and in continuous-wave mode at the apex. The peak instantaneous systolic pressure drop from right ventricle to atrium was calculated from the peak signal velocity of the tricuspid regurgitant signal by a simplified Bermoulli equation. The final estimation of PAP was calculated by adding the patient's jugular pressure. Elevated PAP in this study was defined as PAP > 35 mm Hg. If no tricuspid regurgitation could be found, PAP was regarded as normal.

Plasma ADM assay. Assay of plasma ADM has been described[14]. Plasma immunoreactivity of ADM was determined by a commercial radioimmunoassay kit (Peninsula Laboratories, Belmont, CA, USA). Three milliliters of plasma samples were acidified with 0.75 ml of 2 mol/l hydrochloric acid and centrifuged at 1500 g for 10 min. Supernatants were loaded onto Sep-Pak C18 cartridges (Waters Associates, Milford, MA, USA) that were activated with 100% methyl alcohol and double-distilled deionized water. Cartridges were subsequently washed twice with 3 ml of 0.1% trifluoroacetic acid (TFA) and eluted with 3 ml of 60% acetonitrite in 0.1% TFA. The eluates were then dried under vacuum overnight and resuspended in 250 ml of radioimmunoassay buffer. 100 µl of standard ADM or assay sample were incubated overnight at 4°C with 100 µl of rabbit anti-ADM antiserum. 100 µl of ^{125}I-ADM tracer was added to each tube and incubated another 24 h. Using a goat anti-rabbit antiserum, antibody-bound ADM was precipitated and the radioactivity was measured in a gamma counter. A standard curve was constructed using serial dilutions of freshly reconstituted synthetic human ADM. The plasma level of ADM was expressed as pmol/l.

Statistical analysis. Values quoted here are expressed as mean ± standard deviation (median), unless otherwise specified. Analysis was performed using SPSS 13.0 software (SPSS, Chicago, IL, USA). As plasma levels of ADM were not normally distributed, a Mann-Whitney U test was used to compare ADM levels between SSc patients and healthy controls. Kruskal-Wallis test was done for comparisons involving more than 2 groups, and Mann-Whitney U tests with Bonferroni correction were used subsequently to identify groups with statistical differences. A significance level of $p < 0.05$ was used for all analyses.

RESULTS

Sixty-two SSc patients and 21 healthy controls were recruited. All subjects were ethnic Southern Chinese. The demographic characteristics, vascular manifestations, and medications of SSc patients at the time of study are shown in Table 1. There were 14 with dSSc and 48 with lSSc. The age of these patients was 52.1 ± 13.7 years and their duration of followup was 11.4 ± 9.2 years.

ADM levels of SSc patients and controls. Plasma ADM level was not found to be related to sex ($p = 0.23$) or age ($p = 0.82$) in SSc patients. It was not associated with early disease within 3 years from onset of symptoms ($p = 0.56$) and did not correlate with duration of disease ($p = 0.78$). ADM levels in SSc patients [14.4 ± 19.3 pmol/l (median 9.2)] were not different from controls [9.6 ± 6.1 pmol/l (median 7.9); $p = 0.96$]. There was no difference in ADM level between SSc clinical subsets [for dSSc 16.5 ± 27.7 pmol/l (median 8.5) and for lSSc 13.7 ± 16.4 pmol/l (median 9.7); $p = 0.99$]. There was no relation between plasma ADM levels and presence of anticentromere ($p = 0.73$) or anti-Scl70 antibodies ($p = 0.76$) or ILD ($p = 0.63$).

ADM levels and pulmonary hypertension. Elevated PAP was found in 11 (22.9%) lSSc and 4 (28.6%) dSSc patients in this cohort. The PAP of these patients was 46.5 ± 11.2 (range 37–74) mm Hg. SSc patients with elevated PAP were found to have higher ADM levels [17.9 ± 13.9 pmol/l (median 13.9)] than those patients with no elevation in PAP [13.2 ± 21.0 pmol/l (median 7.2); $p = 0.01$] and controls [9.6 ± 6.1 pmol/l (median 7.9); $p = 0.04$]. The levels of ADM in SSc patients without elevated PAP and in controls were not different ($p = 0.43$). ADM levels were found not to correlate with the level of PAP in SSc patients ($p = 0.24$). Figure 1 shows plasma ADM levels in controls, SSc patients with no elevated PAP, and SSc patients with elevated PAP, and the latter patients with and without concomitant ILD.

Among the 15 patients with elevated PAP, lung function tests were performed in 14 because one patient had significant microstomia that prevented occlusion of the mouthpiece. By lung function criteria, 5 (35.7%) patients had isolated elevations in PAP, whereas 9 (64.3%) had concomitant ILD. The ADM level in patients who had isolated elevation in PAP [21.9 ± 14.2 pmol/l (median 24.7)] was not different from patients who had concomitant ILD [11.7 ± 3.6 pmol/l (median 12.0); $p = 0.19$]. Taking into consideration data from the patient who failed the lung function test but who was known to have ILD from previous high resolution computed tomography scan of thorax, there was no difference in ADM level between these 2 subsets of patients ($p = 0.21$). No relation was found between ADM level and New York Heart Association (NYHA) functional classes among patients with elevated PAP ($p = 0.26$).

ADM level and Raynaud's phenomenon. RP was found in most patients (91.1%) in the cohort, but severe RP was found in 38.7% (n = 24) of patients who required ganglion-block procedure or iloprost infusion (n = 9) and/or had digital infarction or resorption (n = 20). Patients with severe RP were found not to differ in plasma ADM level [19.8 ± 27.5 pmol/l (medi-

Table 1. Demographic characteristics and vascular outcomes of patients with SSc.

	Patients with SSc, n = 62	
	Mean ± SD (range)	No. (%)
Demographic characteristics		
Age at study, yrs	52.1 ± 13.7 (23–83)	
Age at onset, yrs	41.6 ± 15.0 (15–81)	
Duration of disease, yrs	11.4 ± 9.2 (1–40)	
No. of patients with early disease (< 3 yrs since diagnosis)		8/62 (12.9)
Female:male ratio		58:4
SSc subset; diffuse:limited		14:48
Serology		
Antinuclear antibodies		57/62 (91.9)
Anti-Scl70 antibodies		22/62 (35.5)
Anticentromere antibodies		8/62 (12.9)
Vascular outcomes		
Raynaud's phenomenon		57/62 (91.9)
Severe Raynaud's phenomenon		24/62 (38.7)
Digital infarct or resorption		20/24 (83.3)
Ganglion block		2/24 (8.3)
Iloprost infusion		7/24 (29.2)
Elevated pulmonary arterial pressure (> 35 mm Hg),	46.5 ± 11.2 (37–74)	15/62 (35.5)
ILD by lung function measures*		9/14 (64.3)
NYHA functional class		
Class I		3/15 (20)
Class II		6/15 (40)
Class III		5/15 (33.3)
Class IV		1/15 (6.7)
Treatment at the time of study		
Calcium channel-blocker		38/62 (61.3)
Prednisolone		22/62 (35.5)
Penicillamine		18/62 (29.0)

* Lung function test was performed in 14 SSc patients because one patient had significant microstomia secondary to sclerodermatous skin changes.

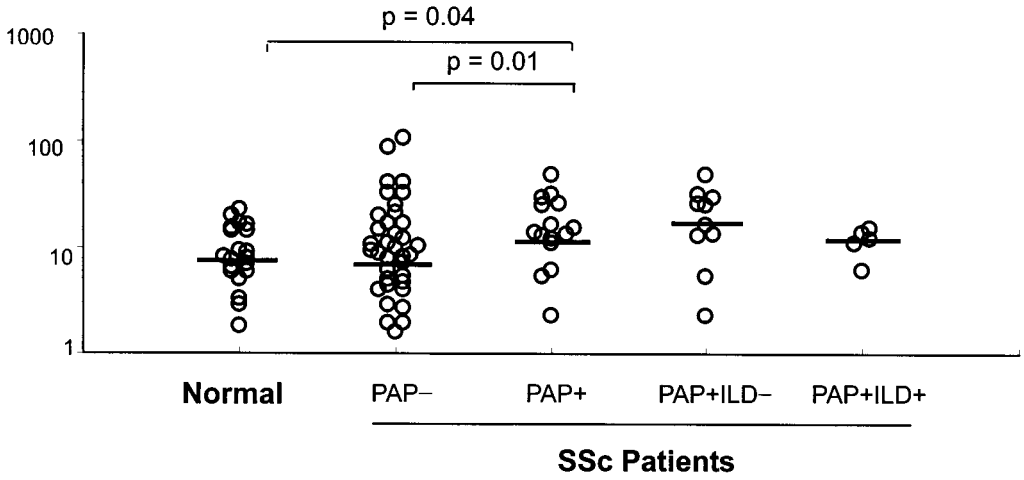

Figure 1. Plasma adrenomedullin levels in healthy controls, SSc patients with (+) and without (–) elevated pulmonary arterial pressure (PAP), and SSc patients with elevated PAP in the presence (+) and absence (–) of concomitant interstitial lung disease (ILD). SSc patients with elevated PAP had higher plasma ADM levels than those without and controls. There was no difference in plasma ADM levels between patients who had isolated elevation in PAP and those whose elevated PAP was associated with ILD.

an 11.9)] from those who did not have this complication [10.9 ± 10.5 pmol/l (median 8.7); p = 0.40] and from controls (p = 0.75).

SSc patients with both severe RP and elevated PAP were found to have the highest ADM levels. Kruskal-Wallis test revealed statistical differences in ADM levels between patients who had neither severe RP nor elevation in PAP (Group 1, n = 31), patients who had severe RP alone (Group 2, n = 14), patients who had elevated PAP alone (Group 3, n = 7), and those who had both severe RP and elevated PAP (Group 4, n = 10) (p = 0.004; Figure 2). The levels of ADM in these 4 groups of patients were 10.2 ± 10.8 (median 8.0), 7.9 ± 10.9 (median 4.2), 14.1 ± 9.0 (median 12.0), and 36.6 ± 35.0 pmol/l (median 22.3), respectively. Mann-Whitney U tests with Bonferroni correction showed statistical differences of ADM levels in patients who had both severe RP and elevated PAP compared to patients who had neither of these vascular manifestations (p = 0.006) and those who had severe RP alone (p = 0.006). There were no differences in ADM levels between Groups 1 and 2 (p = 1.00), Groups 1 and 3 (p = 1.00), Groups 2 and 3 (p = 0.34), and Groups 3 and 4 (p = 0.65).

ADM level and medications. Patients who were receiving calcium channel-blocker were found not to have different ADM levels (15.1 ± 22.8 pmol/l, median 8.1) compared to those who were not (13.2 ± 12.0 pmol/l, median 10.1) (p = 0.58). ADM level was found not to be related to use of penicillamine (p = 0.54) or prednisolone (p = 0.79).

DISCUSSION

Our study demonstrated an association between plasma ADM level and vascular manifestations in a larger cohort of patients with SSc. ADM level was suggested to be elevated in a case series of patients who had primary and secondary RP and in SSc patients with pulmonary hypertension[11,12]. Our study confirmed these findings and showed increased ADM levels in SSc patients with elevated PAP compared to those without and to controls. Even higher ADM levels were found in patients who had both vascular manifestations. ADM levels among SSc patients who had elevated PAP were found not to be different comparing those with and without ILD.

One major limitation of our study was the lack of confirmation of pulmonary hypertension by right-cardiac catheterization. Doppler echocardiography has, nevertheless, been shown to be a reliable noninvasive method[15]. Our results were thus expressed as elevated PAP (PAP > 35 mm Hg) instead of pulmonary hypertension. Indeed, elevated ADM has also been described in primary pulmonary hypertension and pulmonary hypertension secondary to chronic thromboembolism[16,17]. A previous study reported higher ADM level with progressively worse NYHA functional class in cases of congestive heart failure[18], but we did not observe any relationship between ADM level and NYHA functional class among our patients with elevated PAP.

ADM has been found to have higher potency in vasodilation than nitroprusside[19]. In view of its strong vasodilatory

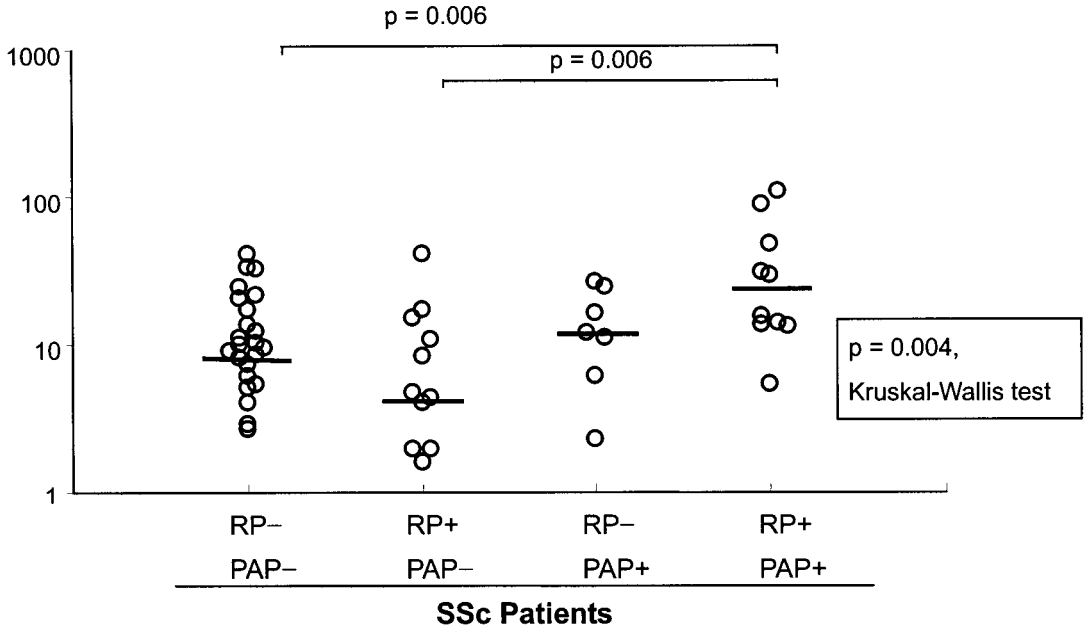

Figure 2. Plasma adrenomedullin levels in different subsets of SSc patients in regard to the presence (+) and absence (–) of severe Raynaud's phenomenon (RP) and elevated pulmonary arterial pressure (PAP). Patients who had both severe RP and elevated PAP had significantly higher plasma ADM levels than patients who had neither manifestation and those who had severe RP only.

effect, increased ADM in patients with elevated PAP may occur as a compensatory mechanism under conditions of increased pulmonary vascular resistance. ADM-specific binding sites have been identified in lung tissues in animals[20,21]. Elevated ADM levels in SSc patients with pulmonary hypertension has been suggested to antagonize the potent vasoconstrictive actions of endothelin-1[12]. ADM has been shown to decrease PAP in the presence of thromboxane A_2 agonist[22], but not on resting arterial pressure of the pulmonary vascular bed in an animal model[23]. It was also found to exhibit antiproliferative effects on pulmonary artery smooth-muscle cells in a paracrine fashion[24]. Indeed, aerosolized[25-27] and infused[28] ADM have been shown in animal studies to reduce mean PAP significantly and to alleviate symptoms of RP in patients with SSc[29].

Unlike previous reports, our findings did not reveal any relation between severe RP and elevated ADM level. Patients who had concomitant severe RP and increase in PAP were, however, found to have higher ADM levels than those who had increased PAP alone, although the difference was not statistically significant. The lack of satisfactory clinical or laboratory measures to indicate severity of RP contributed to one of the limitations of our study. In this regard, we chose to use "irreversible" ischemia to ensure that patients with the most severe vascular problems were evaluated. On the other hand, the lack of increase in ADM expression in severe RP could be secondary to extensive endothelial damage in some patients. As the vascular wall has been suggested to be the major site for production of ADM and that regulation of peripheral and pulmonary circulations are postulated to be similar, it is possible that expression of ADM is higher and more readily detectable given the larger vasculature involved in the pulmonary arterial system. Further, ADM levels may also be influenced by different levels of inflammatory cytokines[2,9] due to variable disease activity[30] as well as the confounding effect of drug treatment in SSc.

Our study demonstrated elevated plasma levels of ADM in SSc patients with elevated PAP. Future studies of larger scale need to be performed to investigate the source of ADM, the interaction between ADM and endothelin-1 in pulmonary hypertension and RP, the prognostic implications of ADM, and a therapeutic role for ADM in these conditions.

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