EXTENDED REPORT

Reduced circulating levels of angiotensin-(1–7) in systemic sclerosis: a new pathway in the dysregulation of endothelial-dependent vascular tone control

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Objective: Systemic sclerosis (SSc) impairs endothelium-dependent vasodilatation. Among angiotensin I (Ang I)-derived compounds, vasoconstrictor angiotensin II (Ang II) and vasodilator angiotensin-(1–7) (Ang-(1–7)), cleaved from ACE and neutral endopeptidase (NEP) 24.11, respectively, play an important role in vascular tone regulation. Ang-(1–7) may act independently or by activating other vasodilating molecules, such as nitric oxide (NO) or prostaglandin I2 (PGI2). Our aim was to assess, in patients with SSc, circulating levels of Ang I, Ang II and Ang-(1–7), with their metabolising enzymes ACE and NEP, and levels of NO and PGI2, and to correlate them to the main characteristics of SSc.

Methods: Levels of Ang I, Ang II, Ang-(1–7), NEP, ACE, NO and PGI2 were measured in 32 patients with SSc, who were also assessed for humoral and clinical characteristics, and 55 controls.

Results: Plasma Ang I, Ang II and Ang-(1–7) levels were lower in patients with SSc than in controls ($p<0.001$ in all cases). When Ang II and Ang-(1–7) levels were expressed as a function of the available Ang I, lower Ang-(1–7) levels in patients with SSc than in controls were confirmed ($p<0.001$), while no difference was found for Ang II levels. In patients with SSc, the Ang II/Ang-(1–7) ratio indicated a prevalence of Ang II over Ang-(1–7), while in controls Ang-(1–7) was prevalent ($p<0.001$). Levels of ACE, NEP, NO and PGI2 were lower in patients with SSc than in controls ($p<0.05$ in all cases).

Conclusion: In patients with SSc, prevalence of the vasoconstricting Ang II over the vasodilator Ang-(1–7) suggests a dysfunction of the angiotensin-derived cascade that may contribute to dysregulation of vascular tone.

Systemic sclerosis (SSc) is a connective tissue disease characterised by microvascular alterations, perivascular inflammation, and excessive accumulation of collagen resulting in skin and internal organ fibrosis. In SSc, the microvascular system is heavily impaired and the activation/damage of endothelial cells (EC) is considered as a pivotal event in the pathogenesis of the disease.[1] EC play a critical role in the control of vascular tone by releasing vasorelaxing and vasoconstricting substances that interact with microvascular smooth muscle cells.[2] The alteration of EC function creates an imbalance between vasoactive substances, leading to the loss of vascular tone control both of endothelium-dependent and endothelium-independent vasodilatation.[2]

ACE, a metallo-carboxypeptidase produced in large quantity by the pulmonary endothelium, mediates the conversion of angiotensin I (Ang I) to angiotensin II (Ang II) and inactivates bradykinin (BK) and substance P (SP). In SSc, the derangement of EC function induces a decrease in endothelial ACE production,[3 4] suggesting that the reduction of ACE activity may represent a reliable and sensible marker of EC injury.[4]

Neutral endopeptidase (NEP), or enkephalinase, is a metallo-endoprotease (Zn^{2+}) produced by fibroblasts[5] and EC[6] that cleaves Ang I into angiotensin-(1–7) (Ang-(1–7)). It also regulates several physiopathological processes by cleavage of peptides involved in nociception (enkephalins and SP), vascular tone (SP, calcitonin gene-related peptide and endothelin), chemotaxis (SP and BK), and immune system functions (SP, calcitonin gene-related peptide and BK).[7]

The renin–angiotensin system (RAS) controls blood pressure and fluid homeostasis. Angiotensinogen, produced in the liver and activated by renin, released by the kidney, is transformed into Ang I and, after processing by ACE, is converted to the biologically active Ang II.

From the discovery of other components of the RAS, such as Ang II receptors (AT_1 and AT_2) and Ang-(1–7), the concept of ''tissue RAS'' or ''local Ang-forming system'' extended our understanding from an endocrine system to a paracrine–autocrine system modulating a wide array of functions in heart, vessels, kidneys, brain and endocrine glands.[8]

Ang II may be considered to be a fundamental molecule in the integrated regulation of vascular tone.[10] It exerts a potent constrictive effect on smooth muscle cells acting via its receptors, and also stimulating the proliferation of smooth muscle cells and angiogenesis.[9] The heptapeptide Ang-(1–7) is a bioactive member of RAS derived from the enzymatic processing of Ang I by an ACE-independent pathway, involving different forms of endopeptidases, mainly NEP.[10–12] Ang-(1–7) can be synthesised in the kidney and on the EC surface.[13 14] Several studies have shown that blood vessels are important sites for the synthesis and the biological actions of Ang-(1–7).[13]

Abbreviations: Ang I, angiotensin I; Ang II, angiotensin II; Ang-(1–7), angiotensin-(1–7); BK, bradykinin; dSSc, diffuse systemic sclerosis; EC, endothelial cell; lSSc, limited systemic sclerosis; NEP, neutral endopeptidase; NO, nitric oxide; PGF1α, prostaglandin F1α; PGI2, prostaglandin I2; RAS, renin–angiotensin system; SP, substance P; SSc, systemic sclerosis

Ang-(1–7) is cleaved by ACE,[14] and inhibition of ACE results in elevation of Ang-(1–7) levels.

Ang-(1–7) is a natural vasodilator,[15 16] it counterbalances the haemodynamic actions of Ang II, and it can exert its biological actions directly or indirectly. In particular, it increases the hypotensive and vasodilating effect of BK,[15 17] promotes the release of prostanoids from EC[18] and smooth muscle cells,[19 20] and the release of NO.[21 22] Moreover, Ang-(1–7) inhibits proliferation of vascular smooth muscle cells and EC in vitro and in vivo[21] and opposes the mitogenic effects of Ang II.[23] Thus, Ang-(1–7) is involved in the complex mechanisms regulating the homeostasis of blood vessels by counterbalancing the vasoconstrictive and anti-angiogenic effects of Ang II.

The net of interaction between Ang I, Ang II and Ang-(1–7) is complex. In fact, in the biochemical cascade of RAS, ACE determines both the production of Ang II and the degradation of Ang-(1–7) while NEP, by converting Ang I into Ang-(1–7), regulates the level of Ang I present in the tissues.[13 14] Moreover, another metallo-proteinase, named ACE 2, may have a role in generating Ang-(1–7) from Ang II, thus regulating Ang II tissue levels and increasing the levels of Ang-(1–7)[14] (fig 1).

In SSc, components of the RAS, such as ACE, may be relevant either for pathogenic implications[4] or for therapeutic options,[24] given the outstanding importance of ACE inhibitors in preventing and treating SSc renal crisis. However, as far as we know, no data have been reported regarding Ang-(1–7) in SSc. The aim of the present study was to evaluate the circulating levels of the three angiotensin peptides Ang I, Ang II and Ang-(1–7), as well as of the enzymes involved in their cleavage, ACE and NEP, and to correlate their levels to the main clinical features of SSc.

PATIENTS AND METHODS

Patients

Thirty-two Caucasian non-smoker patients with SSc (4 men and 28 women, 60.21±9.3 years) selected from the outpatient clinic of the Department of Medicine, section of Rheumatology of the University of Florence, Italy, and 55 non-hypertensive (blood pressure, 115±15/78±10 mm Hg), non-smoker healthy controls, not using significant medications (6 men and 49 women, 58.34±8.5 years) participated in the study. All the subjects selected were not affected by dyslipidaemia, obesity or diabetes, nor did they present a history of cardiac ischaemic disease.

Enrolled subjects gave their written informed consent and the study was approved by the local ethical committee. All patients with SSc were under treatment with alprostadil-α-cyclodextrin, calcium channel blockers, topical glyceryl trinitrate, proton pump inhibitors and clebopride, but not steroids, cyclophosphamide and azathioprine, D-penicillamine, methotrexate or other potential disease-modifying drugs. Patients with hypertension and those being treated with ACE inhibitors and angiotensin receptor blockers were excluded from the study. Before sampling, patients were washed out for 20 days from oral vasodilating drugs, and for 45 days from intravenous alprostadil-α-cyclodextrin. In both patients and controls, blood samples were taken from the antecubital vein between 08:00 and 09:00.

For 2 weeks before sampling, patients and controls were submitted to a diet poor in nitrates contained as preservative in the food.

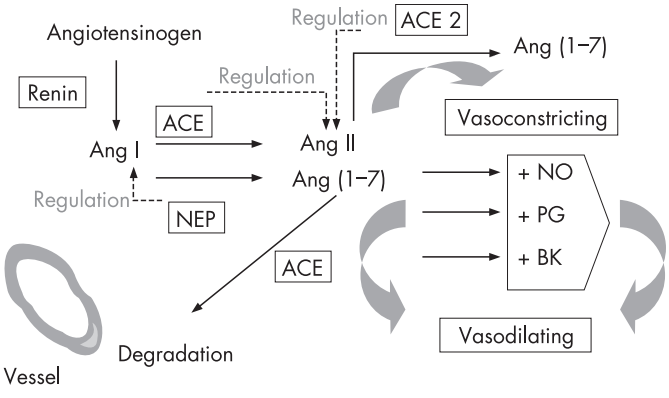

Figure 1 The net of interaction between Ang I, Ang II and Ang-(1–7). Ang I, Angiotensin I; Ang II, angiotensin II; Ang-(1–7), angiotensin 1–7; ACE, angiotensin converting enzyme; ACE 2, angiotensin converting enzyme 2; NEP, neutral endopeptidase; No, nitric oxide; PG, prostaglandin; BK, Bradikynin.

Assessment of patients with SSc

Each patient was evaluated for disease duration (onset of the first non-Raynaud symptom) and subset (diffuse SSc (dSSc) and limited SSc (lSSc)),[25] and assessed according to international guidelines.[26]

Skin and microvascular involvement

At the time of blood sampling, the presence of fingertip ulcers, other skin ulcers (e.g. on heels, legs, elbows and forearms), calcinosis and telangiectasis was recorded. Skin involvement was assessed[27] and scored by the modified Rodnan skin score.[28]

Nailfold video-capillaroscopy

After adaptation to room temperature (20–22°C) for 15 min, nail folds of all 10 fingers were analysed in each patient and examined for the following features: presence of enlarged and giant capillaries, haemorrhages, loss of capillaries, disorganisation of the vascular distribution and ramified/bushy capillaries. According to these features, patients were classified according to Cutolo *et al* [29] in early, active and late pattern.

Lung involvement

Lung involvement was evaluated by forced vital capacity, diffusing lung capacity for carbon monoxide, and high-resolution CT.[30]

Kidney involvement

Kidney involvement was evaluated by assessing serum creatinine concentration, creatinine clearance, urine (24 h) sodium concentration, microalbuminuria and 24 h proteinuria.

Autoantibodies

Antinuclear antibodies (by indirect immunofluorescence on rat liver), anticentromere antibodies (by indirect immunofluorescence on Hep-2 cells and by ELISA for centromere protein antigen) and antitopoisomerase I antibodies (by immunoblot analysis) and rheumatoid factor (by ELISA) were determined.

Evaluation of the circulating levels of Ang I, Ang II, Ang-(1–7), ACE, NEP, NO and PGI2

Plasma concentrations of Ang I, Ang II, Ang-(1–7), ACE and NEP, and serum concentrations of NO and PGI2, were assayed in patients with SSc and in healthy controls. For the assays of all the substances, after 20 min on ice, blood samples were centrifuged at 3000 rpm for 20 min at 4°C, and aliquots of plasma and serum were stored at −80°C until assayed.

For angiotensin peptides, aliquots of plasma were stored at −80°C until assayed. Samples of blood were collected in NH_4-EDTA (25 mM final concentration) and measured by radioimmunoassay as described elsewhere.[31] Briefly, blood taken for determination of angiotensin peptides (10 ml) was poured into

pre-chilled glass tubes that contained 0.5 ml of an inhibitor solution composed of NH_4-EDTA (25 mM), *o*-phenanthroline (0.44 mM) and pepstatin A (0.12 mM final concentration; Sigma), and a cocktail preventing the in vitro metabolism of Ang I during manipulation of the sample. Plasma was extracted using Sep-Pak columns, as previously described.[32 33] The sample was eluted, reconstituted and split for the three radioimmunoassays. Recoveries of radiolabelled angiotensin added to the sample and followed through the extraction were 92% (n = 23). Samples were corrected for recoveries. Ang I was measured using a commercially available kit (Peninsula, Belmont, CA, USA). Ang II was measured using the Alpco (Windham, NH, USA) and Ang-(1–7) was measured using the antibody described previously.[32 33] The minimum detectable levels of the assays were 2.5 pg/tube for Ang-(1–7), 0.8 pg/tube for Ang II and 1.25 pg/tube for Ang I. Values at or below the minimum detectable level of the assay were arbitrarily assigned that value for statistical analysis. The interassay coefficients of variation were 18% for Ang I, 12% for Ang II, and 8% for Ang-(1–7).

NEP plasma levels (pmol/ml/min) were measured by a fluorometric method in which 150 µl of sample obtained from the lyses of the pellet was incubated for 1 h at 37℃ with 10^{-4} M succinyl-alanyl-alanyl-phenylalanyl(7-amido,4-methyl)coumarin (Bachem, Switzerland). Blank values were obtained for each sample by addition of 1 µM thiorphan to the incubation medium, as described.[34] The fluorescence was detected with a fluorometer at a wavelength of 365 nm and emission of 495 nm. The results were corrected per 10^6 cells. Assays were performed in six replicates per well per sample.

ACE levels were evaluated by an ELISA kit (Chemicon International, Temecula, CA, USA; sensitivity 1.2 ng/ml, range 15.6–1000 ng/ml) according to the manufacturer's instructions. The levels of the molecule were evaluated in duplicate serial dilutions of the samples and correlated to a standard curve of ACE.

PGI2 synthesis was measured by using a 6-keto-prostaglandin F1α (PGF1α) electroimmunoassay kit (Cayman Chemical, Ann Arbor, MI, USA; sensitivity 15 pg/ml) according to the manufacturer's instructions. PGF1α levels were evaluated in duplicate serial dilutions of the samples and correlated to a standard curve of PGF1α.

For NO assay, nitrite concentrations in serum were measured by the Griess reaction to estimate total amounts of circulating NO. Circulating nitrite levels were measured by a specific kit (Nitrate/Nitrite Colorimetric Assay kit; Cayman Chemical; sensitivity 2.5 µM for nitrate, 2.0 µM per nitrite) according to the manufacturer's instructions this provided a method for measurement of total nitrate/nitrite concentration in a two-step process. Nitrite production was evaluated in duplicate serial dilutions of the samples and correlated to a standard curve of nitrite.

Statistical analysis

Data were analysed using SPSS 11.5 for Windows. Descriptive statistics are expressed as means (SD) for continuous variables and as number and percentage for categorical variables. Normal distribution of each examined parameter was verified by the Kolmogorov–Smirnoff test. For continuous variables, the two-tailed t test for paired or unpaired data and, when indicated, the Wilcoxon's signed-rank test (paired data) or the U test of Mann–Whitney (unpaired data) were used for analysis between two groups, and the Kruskal–Wallis test (with post hoc Dunn test) for analysis of differences of more than two groups. Fisher's exact test was used for comparison of categorical variables. Non-parametric and parametric correlation analyses were performed with the Spearman's rank correlation test and Pearson test, respectively, and by linear regression analysis. p Values less than 0.05 were considered to be statistically significant.

RESULTS

Patients

Twenty-seven of 32 patients examined had lSSc and five had dSSc.[26] The mean disease duration was 7.9±7.5 years (⩽10 years in 28 patients, ⩽5 years in 16 patients). At capillaroscopy, 11 patients presented an early pattern, and 12 and 9 patients had an active and a late pattern, respectively.[29] The characteristics of the patients are shown in table 1. None of the patients had experienced a scleroderma-induced renal crisis. All patients had normal renal function (creatinine clearance >70 ml/min × 1.73 m^2 of body surface area) and urinary protein excretion in the physiological range (<150 mg/day).

Circulating levels of Ang I, Ang II, Ang 1-7, ACE, NEP, NO and PGI2

In patients with SSc, levels of Ang I were significantly lower than in healthy controls (28.43±14.6 versus 39.71±17.24 pg/ml; p<0.001). Similar results were found for Ang II (13.04±4.89 versus 19.79±7.36 pg/ml; p<0.001) and Ang-(1–7) (8.96±2.04 versus 30.36±22.4 pg/ml; p<0.001). The data are shown in table 2.

When Ang II and Ang-(1–7) levels were expressed as function of the available Ang I (i.e. correcting each value for 100 pg/ml of Ang I), the reduced Ang-(1–7)/Ang I percentage ratio in patients with SSc was confirmed, but no significant difference was detected in Ang II/Ang I percentage ratio

Table 1 Clinical and laboratory characteristics of patients with SSc

Characteristic	SSc (n = 32)
Age (years)	60.21 (9.3)
Height (cm)	158.8 (7.92)
Weight (kg)	62.50 (13.0)
M:F	4:28
Disease duration (years)	7.9 (7.5)
Skin score	12.03 (9.7)
Skin-ulcer positive	5/32
Fingertip-ulcer positive	9/32
Calcinosis positive	5/32
Telangiectasis positive	23/32
Capillaroscopy	
Early	11/32
Active	12/32
Late	9/32
Autoantibodies	
ANA positive	30/32
Scl-70 positive	8/32
ACA positive	18/32
Autoantibody negative	3/33
RF positive	2/32
Anti-DNA positive	2/32
FVC (%)	101.80 (23.13)
DL_{CO} (%)	62.06 (22.82)
Lung HRCT	
Fibrosis positive	15/31
Fibrosis negative	16/31
Blood pressure (mm Hg)	115 (16)/75 (10)
Serum creatinine (mg/dl)	0.84 (0.26)
Creatinine clearance (ml/min)	94.13 (39.10)
Urinary sodium (mEq/l/24 h)	87.27 (41.01)
Microalbuminuria (mg/24 h)	7.743 (5.71)

Values in parentheses are SD.
ACA, anticentromere antibodies; ANA, antinuclear antibodies; Scl-70, antitopoisomerase I; FVC, forced ventilatory capacity; DL_{CO}, diffusing lung capacity for carbon monoxide; HRCT, high-resolution CT.

Table 2 Levels of Ang I, Ang II, Ang-(1–7), ACE, NEP, NO and PGI2 in patients with SSc and controls

	SSc (n = 32)	Controls (n = 55)	p Value
Ang I (pg/ml)	28.08 (14.51)	39.71 (17.24)	<0.001
Ang II (pg/ml)	13.03 (4.81)	19.79 (7.36)	<0.001
Ang-(1–7) (pg/ml)	8.93 (2.01)	30.36 (22.4)	<0.001
ACE (ng/ml)	435.6 (150.0)	594.85 (82.14)	<0.05
NEP (pmol/ml/min)	694.3 (163.6)	1355.3 (190.10)	<0.0001
NO (μM)	19.36 (10.99)	32.39 (7.59)	<0.001
PGI2 (pg/ml)	157.40 (94.78)	296.90 (20.55)	<0.0001

Values are means (SD).

between patients with SSc and controls (table 3). In addition, the Ang II/Ang-(1–7) ratio demonstrated a clear prevalence of Ang II over Ang-(1–7) in patients with SSc (1.515 ± 0.606, median 1.535) while the contrary was observed in controls (0.855 ± 0.468, median 0.754; $p<0.0001$).

Plasma levels of ACE were lower in patients with SSc (426.5 ± 143.4 ng/ml) than in controls (594.85 ± 82.14 ng/ml; $p<0.05$); similar results were found for plasma levels of NEP (694.2 ± 163.6 versus 1355.0 ± 190.10 pmol/ml/min; $p<0.0001$) (see table 2).

In controls, lower NO (19.36 ± 10.99 versus 32.39 ± 7.59 μM; $p<0.001$) and PGI2 (157.40 ± 94.78 versus 296.90 ± 20.55 pg/ml; $p<0.0001$) levels were detected in patients with SSc (see table 2).

Clinical and biological correlation

In patients with SSc, plasma Ang II levels were directly correlated with Ang I ($p = 0.0003$, $r^2 = 0.586$) and to NO ($p = 0.0064$, $r^2 = 0.465$). NEP was directly correlated with NO ($p = 0.032$, $r^2 = 0.465$). Ang II levels were inversely correlated with 24 h urinary sodium excretion ($p = 0.0214$, $r^2 = -0.511$). Age was inversely correlated with PGI2 ($p = 0.003$, $r^2 = -0.500$). Disease duration was directly correlated with Ang I ($p = 0.008$, $r^2 = 0.451$) and Ang II ($p = 0.046$, $r^2 = 0.350$) and inversely with NEP ($p = 0.0011$, $r^2 = -0.542$).

In controls, Ang II and Ang-(1–7) levels had a direct correlation ($p = 0.00396$, $r^2 = 0.373$,) and ACE was directly correlated with NO ($p = 0.002$, $r^2 = 0.45$).

DISCUSSION

We believe that this is the first study to evaluate plasma angiotensin peptide levels and enzymes involved in their cleavage in patients with SSc. Our data show a significant reduction of plasma angiotensin peptides levels, concomitant with the decrease of NEP and ACE levels. This indicates that the levels of the single components of tissue RAS[8] and the whole activity of the system seem to be downregulated in patients with SSc.

In our patients, levels of Ang I, Ang II and Ang-(1–7) were reduced, but, when expressed as function of the available Ang I, Ang II levels in patients with SSc were similar to those of controls, while only Ang-(1–7) levels were reduced. This indicates that, starting from a determined level of Ang I, Ang II is produced in the same quantity from the available substrate in patients with SSc as in controls, while the production of Ang-(1–7) is lower in patients with SSc. Thus, in patients with SSc, the reduction of the vasodilating Ang-(1–7), more than the prevalence of the vasoconstrictor Ang II, may significantly dysregulate the control of vascular tone, thus favouring vasoconstriction.

Table 3 Levels of Ang II and Ang-(1–7) expressed as percentages of Ang I levels

	SSc	Controls	p Value
Ang II (%)	50.9 (20.7)	55.96 (28.06)	NS
Ang 1–7 (%)	36.4 (12.6)	87.85 (70.9)	<0.0001

Values are means (SD).
NS, not significant.

Evidence supports the notion of a functioning RAS localised in the vascular walls. Cultured EC, as well as smooth muscle cells, can generate renin and angiotensin peptides.[35 36] The walls of blood vessels and heart tissues can produce Ang I and Ang II,[37–40] and tissue ACE has a potential regulatory role on local Ang II production.[41] An obligatory role of the endothelium for the conversion of Ang I to Ang II has been claimed[42] and more recently confirmed in a study showing that Ang I release and conversion of Ang I to Ang II was respectively reduced by 90% and abrogated by endothelium denudation.[43]

The downregulation of the RAS system might be explained by the severe and widespread endothelial damage in patients with SSc. In fact, both ACE, which transforms Ang I into the biologically active Ang II, and NEP, which cleaves Ang I into Ang-(1–7) in the circulation,[10–12] are produced in large quantities by the endothelium.

It is well known that the functionality of the system is strictly endothelium dependent. In fact, ACE is present on the membranes of EC,[44] and the production of Ang II from Ang I also depends on the integrity of the EC membranes.[42 43] Moreover, the production of Ang I depends also on de novo vascular synthesis.[37–39 43]

The present data confirm the reduction of circulating ACE in patients with SSc; this has been previously suggested to be a reliable marker of EC injury in patients with SSc.[3 4] The reduction of ACE and Ang I may explain the decrease of Ang II levels. In our patients, plasma levels of Ang II were lower than controls and were normally sensitive to sodium balance, showing a significant inverse correlation with 24 h sodium excretion. Thus, sodium-dependent Ang II regulation seems to be unaffected by the disease.

The reduced circulating Ang II levels found in our patients are in contrast with the results recently obtained by Kawaguchi *et al*[45] who demonstrated higher levels of Ang II in patients with dSSc and no difference in Ang II levels between patients with lSSc and controls. However, in their study, only 11 out of 36 patients with dSSc, characterised by disease duration shorter than 1 year, had higher serum Ang II concentrations. Another confounding factor was that all patients with dSSc, and 24 out of 27 patients with lSSc, were treated with vasodilating drugs at the time of the study and no data were provided on urinary sodium excretion. In our patients with SSc, plasma Ang II levels were shown to depend on sodium balance.

In our patients, the significant reduction of Ang-(1–7), more than Ang II, may be linked with the concomitant reduction of NEP, described in this work for the first time in patients with SSc. Yakomoto *et al* demonstrated that NEP is the most important enzymatic pathway by which Ang-(1–7) is formed from Ang I in the circulation.[10] The EC location of NEP is consistent with its contribution to Ang I metabolism.[6] NEP may be also produced by fibroblasts,[5] and, by cleaving different peptides involved in nociception, vascular tone and immune system functions,[7] it is involved in a wide array of pathophysiological processes. In patients with SSc, the production of NEP by fibroblasts is dysregulated and different between the two subsets of the disease: intracellular NEP activity is higher in the fibroblasts of patients with dSSc than those with lSSc, while its expression on cell surface is lower on the fibroblasts of patients with dSSc compared with those of patients with lSSc.[46]

Although endothelial damage is probably the most important cause of RAS system downregulation and imbalance between Ang II and Ang-(1–7) in patients with SSc, it should be taken into account that some of RAS components may also be produced by vessel smooth muscle cells and fibroblasts[36 46] that, notably overactivated and proliferating in patients with SSc, apart from having a relevant role in the typical ischaemic and fibrotic damage of the disease, may also participate in the dysregulation of RAS.

Although the whole RAS is downregulated, two factors contribute to the dysfunction of vasomotor tone typical of SSc, i.e. a notable reduction of Ang-(1–7) accompanied by a preponderance of Ang II, leading to a shift in the vasomotor control balance toward vasoconstriction. In fact, since Ang II and Ang-(1–7) exert opposite physiological actions on vessels, the net effect depends on the ratio of the two vasoactive substances.

Apart from their action on vascular tone control, Ang II and Ang-(1–7) have opposite effects both on cell proliferation and collagen deposition. Ang II stimulates proliferation of smooth muscle cells and angiogenesis,[9] while Ang-(1–7) inhibits or reverses vascular remodelling and reduces protein incorporation. Indeed, Ang-(1–7) inhibits thrombosis and proliferation of vascular smooth muscle cells[20 21 47] and reduces neointimal formation and smooth muscle cell proliferation after vascular injury.[48] In a rat model of in-stent restenosis, intravenous infusion of Ang-(1–7) attenuates neointimal formation and improves the endothelial-dependent relaxation.[49] Ang-(1–7) also inhibits hypertrophy of cardiomyocytes and hyperplasia in cardiac fibroblasts[50] and inhibits lung cancer cell growth.[51]

In patients with SSc, these data have led to the hypothesis that the downregulation of Ang-(1–7) may be involved in the breakdown of the vessel lumen patency and in the loss of endothelium-dependent vascular tone control, and that it may favour intimal thickening and smooth muscle cell proliferation in the vessel wall.

Ang-(1–7) may also exert its biological activity by stimulating the release of other molecules, such as NO and PGI2, from EC and/or smooth muscle cells.[19 23] Furthermore, these factors are released by Ang-(1–7) in an endothelial-dependent fashion, and thus a damaged endothelium is not a proper substrate for Ang-(1–7) to act. In patients with SSc, the whole scenario also indicates a concomitant reduction of NO and PGI2, consistent with the reduction of Ang-(1–7) and with endothelial damage.

The exact status of NO production in patients with SSc is confusing, as both increased and decreased circulating total nitrate levels and production have been reported.[52 53] Impaired basal levels of NO, as in our case, may contribute to the development of SSc vasculopathy by increasing vasospasm, platelet aggregation, upregulation of adhesion molecules, and increasing vascular thickness.[52]

Low PGI2 levels are in line with the imbalance between the vasodilating PGI2 and the vasoconstricting thromboxane A_2 shown in patients with Raynaud phenomenon and pulmonary vasospasm secondary to mixed tissue connective disease or SSc.[54]

In conclusion, a downregulation of RAS components is present in patients with SSc, resulting in the prevalence of the vasoconstricting Ang II over the vasodilating Ang-(1–7). The dysregulation of RAS components may contribute to impair the alteration endothelium-dependent vasodilatation, thus amplifying the vasoconstricting burden in SSc vessels.

Future studies on angiotensin peptides and new angiotensins are needed to understand whether these substances may be considered markers of endothelial injury or whether they may become potential targets of the treatment in order to restore endothelial-dependent tone control in SSc.

The exogenous administration of Ang-(1–7) might also become a new potential treatment to restore tone control and smooth cell proliferation in patients with SSc.

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REFERENCES

1 **Kahaleh B**, Meyer O, Scorza R. The assessment of the patient with systemic sclerosis. Assessment of vascular involvement. *Clin Exp Rheumatol* 2003;**21 (3 Suppl 29)**:S9–14.
2 **Matucci Cerinic M**, Generini S, Pignone A. New approaches to Raynaud's phenomenon. *Curr Opin Rheumatol* 1997;**9**:544–54.
3 **Pignone A**, Generini S, Matucci-Cerinic M. Prostaglandin E1 restores the levels of vWF and ACE in chronic critical limb ischemia in systemic sclerosis. *Clin Exp Rheumatol* 2001;**19**:358–9.
4 **Matucci Cerinic M**, Jaffa A, Kahaleh B. Angiotensin converting enzyme: an in vivo marker of endothelial injury. *J Lab Clin Med* 1992;**120**:428–33.
5 **Lorkowski G**, Zijderhand-Bleekemolen JE, Erdos EG, von Figura K, Hasilik A. Neutral endopeptidases 24-11(enkephalinase). Biosynthesis and localization in human fibroblasts. *Biochem J* 1987;**248**:345–50.
6 **Llorens-Cortes C**, Huang H, Vicart P, Gasc JM, Paulin D, Corvol P. Identification and characterization of neutral endopeptidase in endothelial cell from venous and arterial origin. *J Biol Chem*, 1992 Jul 15, **267**:14012–8.
7 **Schaible HG**, Del Rosso A, Matucci-Cerinic M. Neurogenic aspects of inflammation. *Rheum Dis Clin North Am* 2005;**31**:77–101.
8 **Chappell MC**, Allred AJ, Ferrario CM. Pathways of angiotensin-(1–7) metabolism in the kidney. *Nephrol Dial Transplant* 2001;**16(Suppl 1)**:22–6.
9 **Cody RJ**. The integrated effects of angiotensin II. *Am J Cardiol* 1997;**79**:9–11.
10 **Yamamoto K**, Chappell K, Brosnihan KB, Ferrario CM. In vivo metabolism of angiotensin I by neutral endopeptidase (EC3.4.24.11) in spontaneously hypertensive rats. *Hypertension* 1992;**19**:1201–11.
11 **Chappell MC**, Gomez MN, Pirro NT, Ferrario CM. Release of angiotensin-(1–7) from the rat hindlimb: influence of angiotensin-converting enzyme inhibition. *Hypertension* 2000;**35**:348–52.
12 **Iyer SN**, Ferrario CM, Chappell MC. Angiotensin-(1–7) contributes to the antihypertensive effects of blockade of the renin–angiotensin system. *Hypertension* 1998;**31**:356–61.
13 **Santos RAS**, Campagnole-Santos MJ, Andrade Santos SP. Angiotensin-(1–7): an update. *Regul Pept* 2000;**91**:45–62.
14 **Ferrario CM**, Trask AJ, Jessup JA. Advances in the biochemical and functional roles of angiotensin converting enzyme 2 and angiotensin-(1–7) in the regulation of cardiovascular function. *Am J Heart Physiol Heart Circ Physiol* 2005;**289**:H2281–90.
15 **Brosnihan KB**, Li P, Ferrario CM. Angiotensin-(1–7) dilates canine coronary arteries through kinins and nitric oxide. *Hypertension* 1996;**27**:523–8.
16 **Benter IF**, Diz DI, Ferrario CM. Cardiovascular actions of angiotensin-(1–7). *Peptides* 1993;**14**:679–84.
17 **Paula RD**, Lima CV, Britto RR, Campagnole-Santos MJ, Khosla MC, Santos RAS. Potentiation of the hypotensive effects of bradykinin by angiotensin-(1–7) related peptides. *Peptides* 1999;**20**:493–500.
18 **Jaswal N**, Diz DI, Chappell MC, Khosla MC, Ferrario CM. Alterations in prostaglandin production in spontaneously hypertensive rats smooth muscle cells. *Hypertension* 1993;**21**:905–9.
19 **Muthalif MM**, Benter IF, Uddin MR, Harper JL, Malik KU. Signal transduction mechanism involved in angiotensin-(1–7)-stimulated arachidonic acid release and prostanoid synthesis in rabbit aortic smooth muscle cell. *J Pharmacol Exp Ther* 1998;**284**:388–98.
20 **Jaiswal N**, Jaiswal RK, Tallant EA, Diz DI, Ferrario CM. Alterations in prostaglandin production in spontaneously hypertensive rat smooth muscle cells. *Hypertension* 1993;**21**:900–5.
21 **Freeman EJ Chisolm GM**, Ferrario CM, Tallant EA. Angiotensin-(1–7) inhibits vascular smooth muscle cells growth. *Hypertension* 1996;**28**:104–8.
22 **Seyedi N**, Xu X, Nasjletti A, Hintze TH. Coronary kinin generation mediates nitric oxide release after angiotensin receptor stimulation. *Hypertension* 1995;**26**:164–70.

23 **Machado RD**, Santos RA, Andrade SP. Opposing actions of angiotensins on angiogenesis. *Life Sci* 2000;**66**:67–76.
24 **Steen VD**, Costantino JP, Shapiro AP, Medsger TA Jr. Outcome of renal crisis in systemic sclerosis: relation to availability of angiotensin converting enzyme (ACE) inhibitors. *Ann Intern Med* 1990;**113**:352–7.
25 **LeRoy EC**, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, *et al.* Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988;**15**:202–5.
26 **The assessment of the patient with systemic sclerosis**. *Proceedings of a symposium, 7–9 February 2002, Portonovo, Italy, Clin Exp Rheumatol* 2003;**21(Suppl 29)**:S2–56.
27 **Akesson A**, Fiori G, Krieg T, van der Hoogen FHJ, Seibold JR. Assessment of skin, joint and muscle involvement. *Clin Exp Rheumatol* 2003;**21(Suppl 29)**:55–8.
28 **Clements P**, Lachenbruch P, Seibold J, White B, Weiner S, Martin R, *et al.* Inter and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. *J Rheumatol* 1995;**22**:1281–5.
29 **Cutolo M**, Sulli A, Pizzorni C, Accardo S. Nailfold videocapillaroscopy assessment of microvascular damage in systemic sclerosis. *J Rheumatol* 2000;**27**:155–60.
30 **Matucci-Cerinic M**, D'Angelo S, Denton CP, Vlachoyiannopoulos P, Silver R. Assessment of lung involvement. *Clin Exp Rheumatol* 2003;**21(3 Suppl 29)**:S19–23.
31 **Suzuki H**. Non renal factors contribute to plasma and cerebrospinal fluid angiotensin II. *Hypertension* 1986;**8(Suppl I)**:I95–100.
32 **Senanayake PD**, Moriguchi A, Kumagai H, Ganten, Ferrario CM, Brosnihan KB. Increased expression of angiotensin peptides in the brain of transgenic hypertensive rats. *Peptides* 1994;**15**:919–26.
33 **Nakamoto H**, Ferrario CM, Fuller DB, Robaczwski DL, Winicov E, Dean RH. Angiotensin-(1–7) and nitric oxide interaction in renovascular hypertension. *Hypertension* 1995;**25**:796–802.
34 **Spillantini MG**, Geppetti P, Fanciullacci M, Michelacci S, Lecomte JM, Sicuteri F. In vivo enkephalinase inhibition by acetorphan in human plasma and CSF. *Eur J Pharmacol* 1986;**125**:147–50.
35 **Kifor I**, Dzau VJ. Endothelial rennin–angiotensin pathway: evidence for intracellular synthesis and secretion of angiotensins. *Circ Res* 1987;**60**:422–8.
36 **Re RN**, Fallon JT, Dzau VJ, Quay S, Haber E. Renin synthesis by canine aortic smooth muscle cells in culture. *Life Sci* 1982;**30**:99–106.
37 **Campbell DJ**. The site of angiotensin production. *J Hypertens* 1985;**3**:199–207.
38 **Admiraal PJJ**, Derkx FHM, Danser AHJ, Pieterman H, Schalekamp MADH. Metabolism and production of angiotensin I in different vascular beds in subjects with hypertension. *Hypertension* 1990;**15**:44–55.
39 **Danser AHJ**, Koning MMG, Admiraal PJJ, Sassen LMA, Derkx FHM, Verdouw PD, Schalekamp MADH. Productions of angiotensins I and II at tissue sites in intact pigs. *Am J Physiol Heart Circ Physiol* 1992;**263**:H429–H437.
40 **Dzau VJ**, Re RN. Tissue angiotensin system in cardiovascular medicine. *Circulation* 1994;**89**:493–8.
41 **Muller DN**, Luft FC. The rennin–angiotensin system in the vessel wall. *Basic Res Cardiol*, 1998;**93**(Suppl 2), 7–14.
42 **Gohlke P**, Bünning P, Unger T. Distribution and metabolism of angiotensin I and II in the blood vessel wall. *Hypertension* 1992;**20**:151–7.
43 **Hilgers KF**, Veelken R, Müller DN, Kohler H, Hartner A, Botkin SR, Stumpf C, Schmieder RE, Ariel Gomez R. Renin Uptake by the endothelium mediates vascular angiotensin formation. *Hypertension* 2001;**38**:243.
44 **Igic R**, Behnia R: Properties and distribution of angiotensin I converting enzyme. *Curr Pharm Des* 2003;**9**:697–706.
45 **Kawaguchi Y**, Takagi K, Hara M, Fukasawa C, Sugiura T, Nishimagi E, *et al.* Angiotensin II in the lesional skin of systemic sclerosis patients contributes to tissue fibrosis via angiotensin II type 1 receptors. *Arthritis Rheum* 2004;**50**:216–26.
46 **Matucci-Cerinic M**, Iannone F, Carossino A, Pignone A, Leoncini G, Generini S, *et al.* Discrepant expression of neprilysin on fibroblasts in diffuse systemic sclerosis. *J Rheumatol* 1999;**26**:347–51.
47 **Kucharewicz I**, Pawlak R, Matys T, Pawlak D, Buczko W. Antithrombotic effect of catopril and losartan is mediated by angiotensin (1–7). *Hypertension* 2002;**40**:774–9.
48 **Strawn WB**, Ferrario CM, Tallant EA. Angiotensin (1–7) reduces smooth muscle growth after vascular injury. *Hypertension* 1999;**33**:207–11.
49 **Langeveld B**, van Gilst WH, Tio AR, Zijlstra F, Roks AJM. Angiotensin 1–7 attenuates neointimal formation after stent implantation in the rat. *Hypertension* 2005;**45**:138–41.
50 **Tallant EA**, Howard LT, Dancho B, Gallagher PE. Angiotensin 1–7 reduces hypertrophy in cardiomyocytes and hyperplasia in cardiac fibroblasts. *Hypertension* 2003;**40**:389.
51 **Gallagher PE**, Tallant EA. Inhibition of human lung cancer cell growth by angiotensin 1–7. *Carcinogenesis*, 2004 25, **11**:2045–52.
52 **Matucci Cerinic M**, Kahaleh B. Beauty and the beast. The nitric oxide paradox in systemic sclerosis. *Rheumatology* 2002;**41**:843–7.
53 **Allanore Y**, Borderie D, Hilliquin P, Hernvann A, Levacher M, Lemarechal H, Ekindjian OG, Kahan A. Low levels of nitric oxide (NO) in systemic sclerosis: inducible NO synthase production is decreased in cultured peripheral blood monocyte/macrophage cells. *Rheumatology* 2001;**40**:1089–96.
54 **Sakamoto K**, Houya I, Inoue K, Tanaka M, Suzuki T, Sakamoto Y, Matsuo H. An imbalance in plasma prostanoids in patients with Raynaud's phenomenon and pulmonary vasospasm. *Eur Respir J* 1999;**13**:137–44.