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CONCISE REPORT

Correlation between plasma concentrations of calcitonin gene related peptide and pulmonary pressure in patients with systemic sclerosis

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Objective: To examine plasma levels of calcitonin gene related peptide (p-CGRP) in patients with systemic sclerosis (SSc) and pulmonary hypertension (PH).

Material and methods: Twenty nine patients with SSc, 10 with diffuse form, 18 with limited form and one with overlapping systemic lupus erythematosus were examined. Twelve patients displayed normal systolic pulmonary artery pressure (PAP_{syst}) ≤30 mm Hg and 17 increased PAP_{syst} >30 mm Hg. Eight patients had isolated PH without interstitial lung disease (ILD) and nine had PH and ILD (secondary PH). PAP_{syst} was measured non-invasively by Doppler cardiography. CGRP was determined by radioimmunoassay.

Results: Patients with PH had higher p-CGRP than patients with normal pressure. A positive relation was found between p-CGRP and PAP_{syst} and between p-CGRP and erythrocyte sedimentation rate (ESR), particularly in patients with isolated PH.

Conclusion: In patients with SSc p-CGRP correlates with pulmonary pressure and with ESR. Whether CGRP reflects disease activity or is released secondary to pulmonary vasoconstriction needs to be investigated further.

Systemic sclerosis (SSc, scleroderma) is characterised by autoimmunity, microangiopathy, and fibrosis of the skin and internal organs. Disturbed microcirculation forms the pathophysiological basis for several complications of the disease, such as digital gangrene, ischaemic heart disease, kidney insufficiency, and pulmonary hypertension (PH). PH may be secondary to pulmonary fibrosis or isolated owing to pulmonary vessel involvement. The natural course of PH is not known and the prognosis is poor. Because aggressive treatment in the early phases of PH might be more effective than the treatment of manifest PH, predictive markers are needed. Increased plasma concentration of von Willebrand factor, released from activated endothelial cells, is reported in SSc; and in a small group of patients with isolated PH, we found a correlation between the concentration of plasma von Willebrand factor and pulmonary pressure.¹

Calcitonin gene related peptide (CGRP) is a potent endogenous vasodilator,² which may be involved in the physiological control of blood flow. CGRP is widely distributed in perivascular nerves.³ Bunker and coworkers described a significant reduction of CGRP immunoreactive neurons in the papillary dermis and around capillaries in deeper dermis in the skin of patients with primary Raynaud's phenomenon (RP) or RP associated with SSc.⁴ They suggested a dysfunction of CGRP in the pathophysiology of RP. CGRP-like immunoreactivity is also localised in the lung, in nerve fibres of the airways from trachea to the level of alveoli.⁵ Tjen-A-Looi and coworkers previously showed that exogenous CGRP reduced

Table 1 Clinical characteristics of 29 patients with SSc divided according to pulmonary pressure

Characteristic	PAP _{syst} (mmHg)	
	≤30	>30
Number	12	17
Sex (male/female)	6/6	6/11
Form (dSSc/lSSc)	3/9	7/10
Age (years), median (range)	51 (41–70)	54 (26–74)
Duration (years), median (range)	1.5 (1–6)	9 (1–27)
VC (%p), median (range)	86 (67–120)	76 (36–118)
TlCO (%p), median (range)	81.5 (35–134)	41 (20–84)
Cardiac hypertrophy (n)	2	4
Interstitial lung disease (n)	4	9
Smokers (n)	4	5
Treatment		
Calcium channel blockers (n)	9	15
Steroids (n)	4	10
Immunosuppressive drugs (n)	3	6

PAP_{syst}, Systolic pulmonary artery pressure; VC, vital capacity; TlCO, carbon monoxide transfer factor; %p, % of predicted value.

pulmonary artery pressure in hypobaric hypoxic rats.⁶ They suggested that endogenous CGRP plays an important part in pulmonary pressure homeostasis. The purpose of our study was to determine CGRP in plasma in patients with SSc with special reference to pulmonary pressure.

PATIENTS AND METHODS

Patients

Twenty nine patients (12 men, 17 women) with SSc were included in the study. Ten patients had diffuse cutaneous SSc with truncal skin involvement and 18 had limited cutaneous SSc with skin involvement restricted to the extremities and face.⁷ One had overlapping systemic lupus erythematosus. All patients had RP and organic vessel changes as measured by finger pressure by finger cooling.⁸

Seventeen patients with increased systolic pulmonary artery pressure (PAP_{syst}) were consecutive patients in whom increased PAP_{syst} was noticed when they were assessed for suspected PH. Twelve patients with normal PAP_{syst} were consecutive patients referred to our department during a period when pulmonary pressure was measured in all patients. Nine patients were smokers. Table 1 shows clinical characteristics of the patients.

Abbreviations: CGRP, calcitonin gene related peptide; ESR, erythrocyte sedimentation rate; ILD, interstitial lung disease; PAP_{syst}, systolic pulmonary artery pressure; PH, pulmonary hypertension; RP, Raynaud's phenomenon; SSc, systemic sclerosis; TlCO, carbon monoxide transfer factor; VC, vital capacity

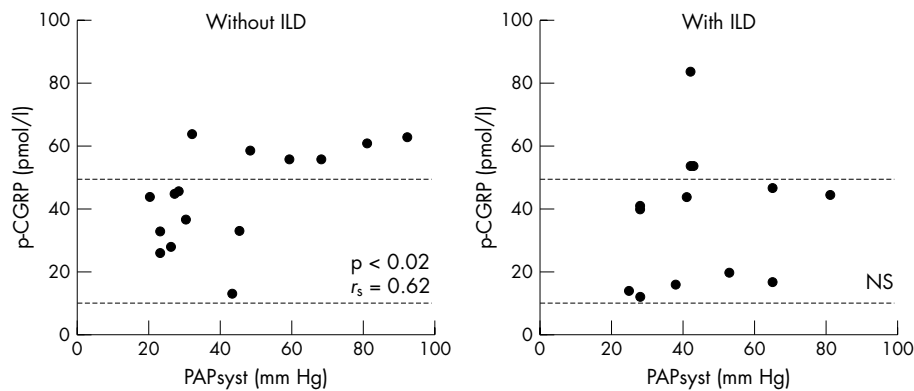


Figure 1 Relation between plasma concentration of calcitonin gene related peptide (p-CGRP) and systolic pulmonary artery pressure (PAPsyst) in 29 patients with SSc, 16 patients without ILD (A) and 13 patients with ILD (B). Mean \pm 3SD for healthy controls is marked with dotted lines.

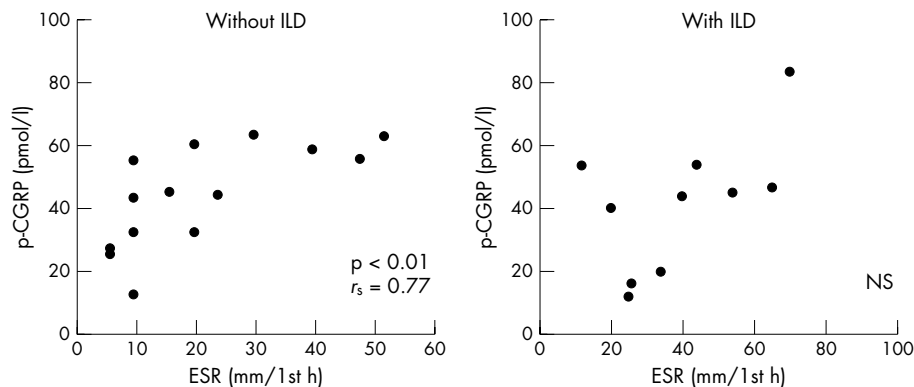


Figure 2 Relation between plasma concentration of calcitonin gene related peptide (p-CGRP) and ESR (mm/1st h) in 24 patients with SSc, 14 patients without ILD (A) and 10 patients with ILD (B).

Methods

Plasma concentrations of CGRP (p-CGRP) were analysed by radioimmunoassay with a rabbit antiserum directed against synthetic CGRP. CGRP in the sample and calibrator competed with [125 I]CGRP for binding to the antibody. CGRP concentrations in the samples and calibrators were in inverse relationship to the volume of bound [125 I]CGRP. The [125 I]CGRP bound to the antibody was separated from the unbound through precipitation with another antibody. Radioactivity in the precipitate was measured with a calibrator curve. Synthetic CGRP (rat) was used as calibrator and [125 I](Tyr 0)-CGRP (rat) as a tracer. The rabbit antiserum (K-8429) consisted of 200 μ l at a final dilution 1/28 000.⁹ The normal value for healthy controls is 30 (10–50) pmol/l (mean \pm 3SD).

The PAPsyst was determined by Doppler cardiography, a non-invasive technique allowing calculation of pressure from the velocity of regurgitant blood flow through the tricuspid valve.¹⁰ All the measurements were evaluated by the same cardiologist. PH was defined as PAPsyst >30 mm Hg. Interstitial lung disease (ILD) and hypertrophy of the heart were diagnosed by standard posteroanterior and lateral chest radiographs. Pulmonary function was assessed by determination of vital capacity (VC) as measured by dry spirometry and by carbon monoxide transfer factor (T_{lco}) by a single breath procedure.

Statistics

Levels of significant differences between the two groups were calculated by the Mann-Whitney U test for unpaired observations. The relations between variables were calculated with Spearman's r_s .

RESULTS

Patients with PH had higher p-CGRP (median 54 pmol/l) than patients with normal PAPsyst (median 35 pmol/l, $p < 0.05$). Eight patients with isolated PH had a median p-CGRP of 58 pmol/l compared with 45 pmol/l in nine patients with PH and ILD (secondary PH), NS.

Among all the patients a positive correlation was found between p-CGRP and PAPsyst ($r_s = 0.43$, $p < 0.05$), whereas no relation was found between p-CGRP and T_{lco} , or between p-CGRP and VC. A correlation was also found between p-CGRP and erythrocyte sedimentation rate (ESR) ($r_s = 0.46$, $p < 0.5$) but not with other biochemical parameters of inflammation (C reactive protein and orosomucoid). When the patients were divided according to the presence or absence of ILD, those without ILD showed a more pronounced correlation between p-CGRP and PAPsyst ($r_s = 0.62$, $p < 0.02$, fig 1A), whereas no relation was found in patients with ILD (fig 1B). Similarly, the relation between p-CGRP and ESR was more pronounced in the subgroup without ILD ($r_s = 0.77$, $p < 0.01$, fig 2A), with no relation in the group with ILD (fig 2B). In comparison with patients without ILD, those with ILD had a higher ESR ($p < 0.05$), lower VC ($p < 0.005$), and lower T_{lco} ($p < 0.05$), but no difference in PAPsyst and p-CGRP was seen between the two groups.

No difference was found in p-CGRP between patients with and without treatment with calcium channel blockers, steroids, or immunosuppressive drugs.

DISCUSSION

A dysregulated neuroendothelial control of vascular tone may explain the microcirculatory impairment in SSc.¹¹ Matucci-Cerinic studied plasma levels of CGRP in 23 cases with limited

cutaneous SSc, and noticed significant reduction of CGRP levels, particularly in patients with long disease duration.¹² He suggested that a persistent inflammatory stimulation of the skin in the early stages of disease induces a release of the neuropeptides, leading to a progressive depletion of the fibres of the peripheral nervous system and, consequently, to an impairment of the control of the local vascular tone. This theory accords well with the results of Bunker and coworkers,⁴ who reported a significant reduction of CGRP immunoreactive neurons in the skin of patients with SSc.

In our study higher plasma levels of CGRP were found in patients with SSc with PH than in patients with normal pressure. A correlation was noted between p-CGRP and PAPsyst in the whole group, but particularly in patients with isolated PH. This might indicate either a mechanism by which CGRP is reflecting disease activity in isolated PH or a CGRP release secondary to pulmonary vasoconstriction. Many patients in this study had a short disease duration which might explain why we did not find decreased p-CGRP as reported by Matucci-Cerinic.¹²

A possible relation between CGRP and inflammation is indicated by several observations. Intradermal injection of CGRP into human skin gives a characteristically prolonged erythematous component, accompanied by an infiltration of polymorphonuclear leucocytes.¹³ CGRP is reported to have a proliferative effect on human endothelial cells.¹⁴ Onuoha and Alpar reported increased p-CGRP in patients with soft tissue injury within 24 hours of injury.¹⁵ In the present study there was a correlation of CGRP with ESR but not with other biochemical parameters. Patients with SSc are known to have a relatively discrete acute phase response, which might explain this discrepancy. ESR, however, is influenced also by immunoglobulins, which are often increased in patients with SSc. The obvious difference in the relation between p-CGRP and ESR in the two subgroups with and without ILD emphasises that the disease mechanisms are different in the two types of PH.

In conclusion, this study showed increased p-CGRP in patients with SSc and PH compared with patients with normal pressure. Further studies are required to clarify whether the increased p-CGRP levels reflect disease activity or whether they are secondary to pulmonary vasoconstriction.

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