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Plasma Endothelin and the Aminoterminal Propeptide of Type III Procollagen (PIIINP) in Systemic Sclerosis

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Forty-four patients with systemic sclerosis and 3 patients with localized scleroderma were investigated for plasma endothelin and aminoterminal propeptide of type III procollagen (PIIINP). Although there was an overlap between plasma levels of endothelin in patients with systemic sclerosis and healthy controls, the mean value of the patients was significantly higher than in controls. Plasma endothelin was normal in all 3 patients with localized scleroderma. The highest levels of plasma endothelin were found in patients with type II and III systemic sclerosis with the largest cutaneous involvement, and in patients with the scleroderma-specific antibodies Scl-70 and anticentromere antibodies. Extremely high values were found in a patient who experienced a renal crisis and in a patient who had her lower leg amputated due to severe vasculitis. A positive correlation was found between plasma endothelin and serum PIIINP. This, together with the fact that in systemic sclerosis the vascular lesions are the earliest, would seem to support the theory that endothelial cell damage could lead to increased secretion of endothelin and subsequent fibrosis in this disease. ***Key words: fibrosis; Scl-70; antinuclear antibodies; localized scleroderma.***

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Endothelins (ETs) are a family of three newly discovered vasoactive peptides mainly synthesized and released by endothelial cells. The effect usually develops slowly but is lasting (1). Renal vessels are particularly sensitive to the vasoconstricting effect of ET. ETs may also contribute to the pathogenesis of vasospasms in systemic sclerosis (SS). It has recently been shown that ET-binding density is higher in microvessels of skin from scleroderma patients (2), and two groups have found elevated plasma ET levels in SS (3, 4). Yamane and co-workers (4) reported higher values in patients with widespread skin involvements than in patients with restricted involvement, affecting the skin distal to wrists and ankles. They also reported an inverted correlation to carbon monoxide diffusing capacity. In a preliminary study on 18 patients with SS (5), we reported that high plasma levels of ET in SS were especially found in the group of patients with the scleroderma antibodies Scl-70 and anticentromere antibodies. Scl-70 antibodies, precipitating antibodies to topoisomerase-I, are unique to SS and occur in about 20% of patients, particularly in those with lung involvement (6).

The present paper contains information on plasma ET in 44 consecutive patients with SS and 3 patients with localized scleroderma and compares ET with serum aminoterminal propeptide of type III procollagen (PIIINP), which has been reported to correlate with involvement of skin and internal organs in SS (7, 8).

PATIENTS AND METHODS

All 44 patients with SS fulfilled the criteria of the American Rheumatism Association (9). Eleven patients suffered from diffuse cutaneous SS (type III). Seventeen had type II limited cutaneous SS, with lesions above the wrists, and 16 type I limited cutaneous SS with no lesions above wrists. Two patients were without treatment when studied; one of these patients had a very progressive disease and later died from renal failure. All remaining SS patients were studied during treatment with D-penicillamine or various immunosuppressive agents, with the exception of one patient who only received colchicine. Two of the 3 patients with localized scleroderma only had topical steroids, while the third patient got D-penicillamine. The treatments are presented in Fig. 1.

All patients were studied with respect to serum creatinine, alkaline phosphatases, and aspartate aminotransferase. Patients with suspected kidney involvement were studied with chrome-EDTA-clearance. Other internal manifestations were diagnosed by lung X-ray, lung function test, echocardiogram and studies on esophageal motility. Patients with suspected joint involvement had an X-ray of these joints, in general

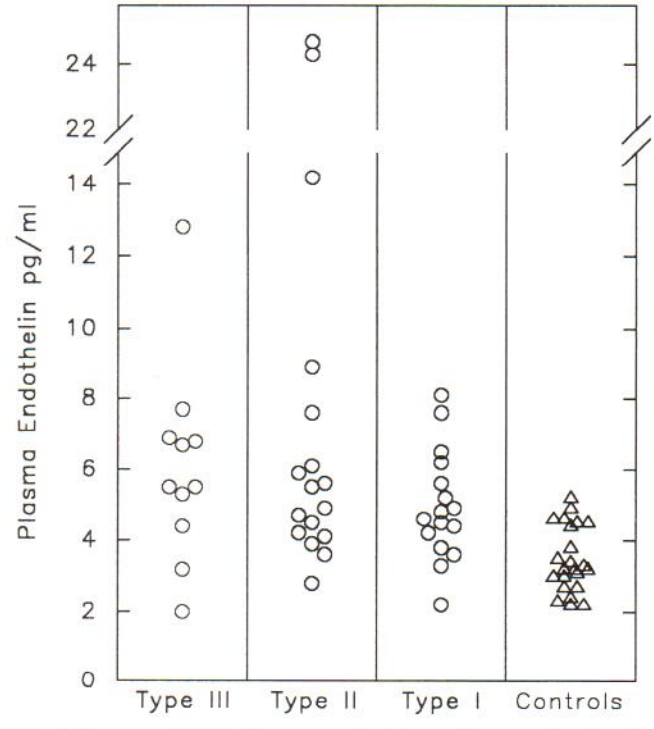

Fig. 1. Plasma endothelin in groups of patients with systemic sclerosis, patients with localized scleroderma and in healthy controls.

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Table I. *Plasma endothelin in patients with systemic sclerosis (SS), localized scleroderma and in healthy controls*

The difference between patients with SS and controls is significant ($p < 0.001$)

Patient group	*n*	Plasma endothelin pg/ml
Type III diffuse	11	6.01 ± 2.8
Type II limited	17	7.97 ± 6.6
Type II & III	27	7.24 ± 5.6
Type I SS limited	16	4.97 ± 1.5
Scl-70 pos.	13	8.54 ± 7.1
Anticentromere AB.pos.	11	6.35 ± 3.5
Anticentromere AB.pos. + Scl-70 pos.	24	7.53 ± 5.8
RNP pos.	7	5.33 ± 1.7
Nuc pos.	9	4.38 ± 1.1
ANA neg.	5	5.08 ± 1.5
All SS patients	44	6.32 ± 4.6
Localized scleroderma	3	3.30 ± 0.3
Controls	23	3.47 ± 0.9

X-ray of the hands. A biliary liver cirrhosis was confirmed in one patient by a liver biopsy.

ET was studied by radioimmunoassay in peripheral venous plasma by the method of the Nichols Institute (Wijchen, Netherlands) as described in detail by Voerman et al. (10). The acidified sample was extracted on a silica C18 cartridge. ET was then determined by a competitive radioimmunoassay using iodinated ET-1 as tracer. The sensitivity of the assay was 1 pg/ml; cross reactivity with ET-2 is 53%, with ET-3 96% and with big-ET 7%. The normal reference range obtained was 2.0–5.3 pg/ml. Serum PIIINP levels were measured by radioimmunoassay based upon the human propeptide (11) with a kit from Orion Diagnostica, Oulunsalo, Finland. The antinuclear antibodies were detected by indirect immunofluorescence on HEp-2 cells by Statens Seruminstitut (Denmark). Anticentromere antibodies were determined by their typical pattern in interphase and metaphase nuclei, and immunodiffusion assays were performed for Scl-70 antigen. The antigens had been manufactured by BioLab (Belgium).

RESULTS

Patients suffering from SS had a higher average plasma ET than controls ($p < 0.001$) (Table I). The 3 patients with localized scleroderma all had normal ET levels. The highest levels of plasma ET were found among patients with type II and type III SS (Fig. 1) and among patients with the antibodies Scl-70 and anticentromere antibodies (Fig. 2).

When comparing 22 patients with decreased carbon monoxide diffusing capacity (< 70% of estimated values) with 17 patients with a normal carbon monoxide diffusing capacity, we found no significant difference. The 2 patients with the highest values were a 72-year-old female (ET: 24.7 pg/ml) with severe vasculitis, who had to have an amputation of the lower leg, and a 62-year-old male (ET: 24.3 pg/ml) who had a renal crisis and later died of renal failure. Both had Scl-70 antibodies. Only 2 of 8 SS patients with cutaneous vasculitis had normal plasma ET. One of these patients with a highly elevated plasma ET (12.8 pg/ml) was receiving an apparently successful treatment with cyclosporin A. Looking for a correlation between ET and PIIINP (Fig. 3), we found a positive correlation with a linear regression ($r = 0.69$, $p < 0.005$).

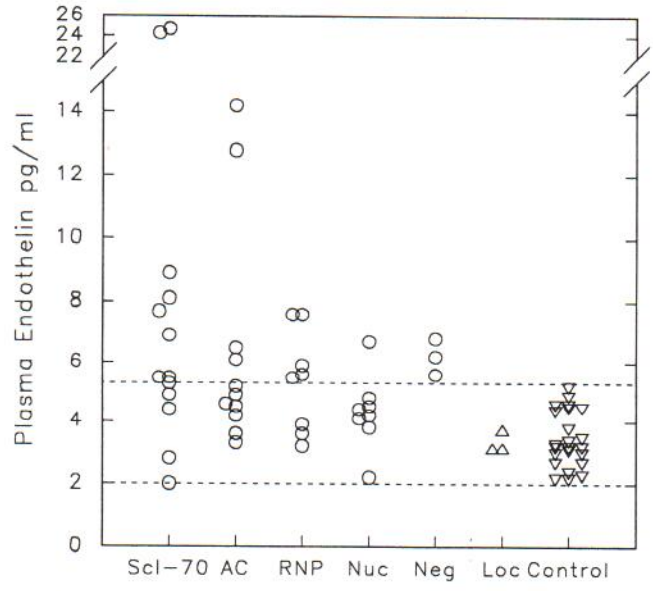

Fig. 2. Plasma endothelin in systemic sclerosis patients with different antinuclear antibodies, patients with localized scleroderma *(Loc)*, and in healthy controls *(Control)*. *AC*, centromere antibodies; *RNP*, ribonuclear protein antibodies; *Nuc*, nucleolar antibodies; *Neg*, negative antinuclear antibody test.

DISCUSSION

The results of our study confirm an increase of plasma ET in patients with SS. The profibrotic action of ET, already suggested by Kahaleh (3), in SS makes it likely that disturbances in the control of ET may contribute to the pathogenesis of the disease. The vascular endothelium is of importance not only to the vascular function, but also to perivascular tissue, and in most patients with SS vascular symptoms such as Raynaud's phe-

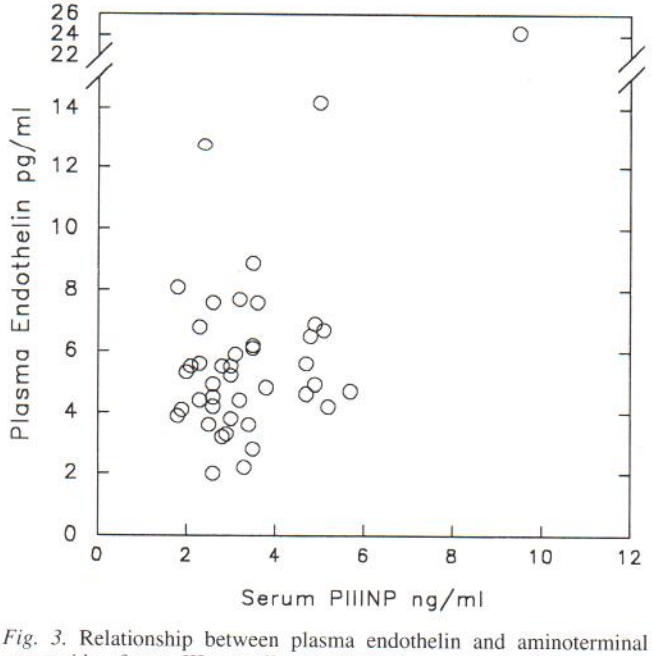

Fig. 3. Relationship between plasma endothelin and aminoterminal propeptide of type III procollagen (PIIINP) in patients with systemic sclerosis.

nomenon are usually the earliest. The correlation found between ET and PIIINP seems to support the theory that endothelial cell damage could lead to increased secretion of ET and subsequent fibrosis.

We have no information on the treatment of the patients reported by Yamane et al. (4); however, we find that our studies are limited by the fact that most of our patients were investigated during treatment with immunosuppressive agents. All of these agents at least may decrease PIIINP (7), while it is more uncertain which influence there would be on ET.

Cyclosporin A is a potent immunosuppressive agent and may improve SS (12). However, it can also induce severe renal crisis, especially in Scl-70-positive SS patients (13), and ET may be released in these cases. Although cyclosporin A stimulates synthesis of ET by human endothelial cells in tissue culture (14), circulating ET has been found independent of cyclosporin A after cardiac transplantation (15). One of our patients with high plasma ET (12.8 pg/ml) was successfully given cyclosporin A therapy, and as a result of this therapy a low PIIINP (3.1 ng/ml) was found.

The present data should be considered preliminary, and we could not find the same inverse correlation between pulmonary involvement with decreased carbon monoxide diffusing capacity and ET as Yamane et al. (4). We agree with these authors that a high plasma ET in a patient with SS may be an indication of a poor prognosis, but longitudinal studies on ET in SS are needed.

REFERENCES

1. Webb D, Haines W. Endothelins come of age. Lancet 1993; 342: 1439–1440.
2. Knock GA, Terenghi G, Bunker CB, Bull HA, Dowd PM, Polak JM. Characterization of endothelin-binding sites in human skin and their regulation in primary Raynaud's phenomenon and systemic sclerosis. J Invest Dermatol 1993; 101: 73–78.
3. Kahaleh M. Endothelin and endothelial-dependent vasoconstrictor in scleroderma. Arthritis Rheum 1991; 34: 978–983.
4. Yamane K, Miyauchi T, Suzuki N, Yuhara T, Akama T, Suzuki H, et al. Significance of plasma endothelin-1 levels in patients with systemic sclerosis. J Rheumatol 1992; 19: 1566–1571.
5. Heickendorff L, Zachariae H, Bjerring P, Halkier-Sørensen L. Plasma endothelin and antinuclear antibodies in systemic sclerosis. Submitted.
6. Cattagio L, Bernstein R, Black C. Serological markers in progressive systemic sclerosis: clinical correlating. Ann Rheum Dis 1983; 42: 23–27.
7. Heickendorff L, Parvez A, Bjerring P, Halkier-Sørensen L, Zachariae H. Serum aminoterminal propeptide of type III procollagen in systemic sclerosis. A follow-up investigation in subclasses and during therapy. Acta Derm Venereol (Stockh) 1991; 71: 185–188.
8. Zachariae H, Bjerring P, Heickendorff L, Møller B, Wallevik K, Angelo H. Photopheresis in systemic sclerosis: clinical and serological studies using markers of collagen metabolism. Acta Derm Venereol (Stockh) 1993; 73: 356–361.
9. Subcommittee for scleroderma criteria of the American Rheumatism Association. Arthritis Rheum 1980; 23: 581–590.
10. Voerman HJ, Stehouwer CD, van Kamp GJ, Strack van Schijndel RJ, Groeneveld AB, Thijs LG. Plasma endothelin levels are increased during septic shock. Crit Care Med 1992; 20: 1097–1101.
11. Risteli J, Niemi S, Trivedi P, Maentausta O, Mowat AP, Risteli L. Rapid equilibrium assay for the aminoterminal propeptide of human type III procollagen. Clin Chem 1988; 34: 715–718.
12. Zachariae H, Halkier-Sørensen L, Heickendorff L, Zachariae E, Hansen HE. Cyclosporin A treatment of systemic sclerosis. Br J Dermatol 1990; 122: 677–681.
13. Zachariae H, Hansen HE, Olsen TS. Hemolytic uremic syndrome in a patient with systemic sclerosis treated with cyclosporin A. Acta Derm Venereol (Stockh) 1992; 72: 307–309.
14. Bunchman T, Brookshire C. Cyclosporin-stimulated synthesis of endothelin by human endothelial cells in tissue culture. Kidney Int 1990; 37: 365.
15. Edwards B, Hunt S, Fowler M, Valantine H, Anderson L, Lerman A. Effect of cyclosporine on plasma endothelin levels in humans after cardiac transplantation. Am J Cardiol 1991; 67: 782–784.