Circulating collagen metabolites in systemic sclerosis. Differences between limited and diffuse form and relationship with pulmonary involvement*

A. Scheja, M. Wildt, F. A. Wollheim, A. Åkesson and T. Saxne

Department of Rheumatology, University Hospital, Lund, Sweden

Abstract

Objective. To study collagen metabolites in systemic sclerosis (SSc) and their relationship with clinical manifestations of the disease.

Methods. Forty-eight SSc patients, 13 with a diffuse form (dcSSc), 23 with a limited form (lcSSc) and 12 with suspected SSc not fulfilling the ACR criteria, and 31 healthy controls were examined. Serum concentrations of aminoterminal type III procollagen peptide (PIIINP), aminoterminal and carboxyterminal type I procollagen peptides (PINP and PICP) and cross-linked carboxyterminal telopeptide of collagen I (ICTP) were determined by radioimmunoassay.

Results. Increased serum concentrations of ICTP were found in SSc patients compared with controls. Distinctly higher levels of ICTP were observed in dcSSc than in lcSSc. High serum ICTP was correlated with skin score and acute phase reactants, and with reduced pulmonary function. Serum PIIINP concentration was elevated in both lcSSc and dcSSc.

Conclusion. Augmented collagen catabolism accompanies the increased collagen synthesis in SSc. Serum ICTP concentration is a marker of this feature and also reflects clinical severity.

Key words: Systemic scleroderma, Collagen metabolites, Collagen catabolism, Procollagen peptide, Telopeptide.

Systemic sclerosis (SSc) is characterized by autoimmunity, microangiopathy and fibrosis of the skin and internal organs. An endothelial lesion is thought to be the primary event in the microangiopathy. An increased production of endothelin-I, an endothelium-derived vasoconstricting peptide with reported profibrotic action [1] may constitute a link between the angiopathy and the fibrosis. Collagen synthesis is increased in SSc fibroblasts [2–4] whereas the degradation of collagen and the amount of immunologically reacting collagenase are reported to be normal [4]. However, Jimenez et al. [5] found a slight but significant increase in the fraction of newly synthesized collagen degraded intracellularly. Furthermore, Stone et al. [6] showed increased levels of urinary cross-linked amino acids in SSc patients, thus indicating increased degradation of mature collagen and elastin. Serum concentrations of procollagen peptides reflecting collagen synthesis have been shown to be increased in SSc and to relate to disease activity [7–9]. This study was undertaken to gain more insight into collagen turnover in early SSc in relation to the involvement of different organs, with particular emphasis on the balance between synthesis and degradation. We present results supporting the presence of increased catabolism as a marker of the severity of SSc.

Materials and methods

Forty-eight consecutive patients referred to our department between January 1995 and July 1996 to be evaluated for systemic sclerosis were included in the study. SSc was confirmed in 36 patients according to the preliminary American College of Rheumatology (ACR) criteria [10]. Twenty-three of these had limited cutaneous systemic sclerosis (lcSSc), with skin sclerosis limited to the extremities and face [11], and 13 had diffuse cutaneous systemic sclerosis (dcSSc) with additional involvement of the skin of the trunk. Twelve patients were classified as having suspected systemic sclerosis (suspSSc) with Raynaud’s phenomenon and sclerodactyly, but did not fulfil the ACR criteria. The onset of the disease was defined as the time when the skin became involved. No patient had been treated with any putat-
Circulating collagen metabolites in scleroderma

Orosomucoid (g) CRP (mg) ESR (mm) – GFR (%) 109 (94
PAPsyst (mm Hg) 36 (20
DLCO (%) 77 (46

Glo
terular filtration rate (GFR) was A marked correlation was found between serum PIIINP and serum ICTP (P < 0.001). No difference in either of the collagen metabolites was found between smoking and non-smoking patients. A marked correlation was found between serum PIIINP and serum ICTP (ρ = 0.80, P < 0.01) and between serum PIIINP and PICP concentrations (ρ = 0.50, P < 0.01). The relationship between serum PICP and ICTP concentrations was less pronounced (ρ = 0.39, P < 0.05) and no relationship was found

Results

Serum-PIIINP was increased in dcSSc and in lcSSc compared with healthy controls (Table 1). Higher levels were seen in dcSSc than in lcSSc (P < 0.01). No significant difference in serum PINP was seen between patients and controls, whereas a moderate decrease in serum PICP concentration was noted in suspSSc (P < 0.05). The serum ICTP concentration was increased both in dcSSc and in lcSSc compared with controls (Table 1). Patients with dcSSc had higher levels than patients with lcSSc (P < 0.001). No difference in either of the collagen metabolites was found between smoking and non-smoking patients.

Statistics

The significance of differences between patients and controls was calculated using the Mann–Whitney test for unpaired samples and correlation between pairs of parameters with Spearman’s ρ.

Table 1. (A) Serum concentrations of collagen peptides of 48 patients with systemic sclerosis divided according to skin involvement, and in 31 healthy controls

<table>
<thead>
<tr>
<th>Disease form</th>
<th>dcSSc</th>
<th>lcSSc</th>
<th>SuspSSc</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>13</td>
<td>12</td>
<td>12</td>
<td>31</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>1/2</td>
<td>1/2</td>
<td>1/2</td>
<td>1/10</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>49 (27–75) ***</td>
<td>56 (18–76) ***</td>
<td>53 (20–76)</td>
<td>47 (22–78)</td>
</tr>
<tr>
<td>PINP (µg/l)</td>
<td>6.0 (3.5–17.0) ***</td>
<td>3.5 (1.5–10.5) ***</td>
<td>2.5 (1.5–4.0)</td>
<td>2.5 (1.0–4.0)</td>
</tr>
<tr>
<td>PICP (µg/l)</td>
<td>40 (26–58)</td>
<td>35 (10–90)</td>
<td>41 (14–68)</td>
<td>43 (19–114)</td>
</tr>
<tr>
<td>ICTP (µg/l)</td>
<td>135 (80–205)</td>
<td>110 (65–215)</td>
<td>100 (65–165)*</td>
<td>130 (55–280)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>Median (range)</td>
<td>Median (range)</td>
<td>Median (range)</td>
<td>Median (range)</td>
</tr>
</tbody>
</table>

(B) Clinical and biochemical characteristics of the 48 SSC patients

<table>
<thead>
<tr>
<th>Disease duration (yr)</th>
<th>Median (range)</th>
<th>Median (range)</th>
<th>Median (range)</th>
<th>≤30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin score (points)</td>
<td>26 (15–36)</td>
<td>5 (2–15)</td>
<td>3 (2–4)</td>
<td>80–120</td>
</tr>
<tr>
<td>VC (%)</td>
<td>97 (61–148)</td>
<td>94 (51–120)</td>
<td>102 (84–130)</td>
<td>80–120</td>
</tr>
<tr>
<td>DLCO (%)</td>
<td>77 (46–111)</td>
<td>77 (40–116)</td>
<td>86 (41–116)</td>
<td>80–120</td>
</tr>
<tr>
<td>PAPsyst (mm Hg)</td>
<td>36 (20–43)</td>
<td>30 (18–65)</td>
<td>28 (24–38)</td>
<td>100–120</td>
</tr>
<tr>
<td>GFR (%)</td>
<td>109 (94–130)</td>
<td>95 (70–136)</td>
<td>103 (93–138)</td>
<td>75–11.2</td>
</tr>
<tr>
<td>Capillary density (loops/mm)</td>
<td>5.1 (2.9–8.3)</td>
<td>4.5 (2.2–8.4)</td>
<td>6.5 (5.4–7.4)</td>
<td>5.0–10.5</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>18 (2–70)</td>
<td>14 (4–70)</td>
<td>18 (2–70)</td>
<td>20</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>6.5 (5–88)</td>
<td>5 (5–32)</td>
<td>5 (5–26)</td>
<td>5</td>
</tr>
<tr>
<td>Orosomucoid (g/l)</td>
<td>0.94 (0.70–1.89)</td>
<td>0.87 (0.42–1.63)</td>
<td>0.83 (0.45–1.48)</td>
<td>0.55–1.05</td>
</tr>
</tbody>
</table>

Difference between patients and controls: *P < 0.05; ***P < 0.001.

ESR = erythrocyte sedimentation rate.

Considerably and disease-modifying drug before sampling. Four patients were under treatment with oral corticosteroids, three of them with ≤5 mg/day and one with 20 mg/day. Nineteen patients were being treated with calcium channel blockers and three with angiotensin converting enzyme inhibitors. Seven patients were smokers, 14 were ex-smokers and 27 were non-smokers. The controls were 31 healthy persons. One control was a smoker, 24 were non-smokers, and in six controls smoking habits were unknown. Gender and age of patients and controls are shown in Table 1.

Aminoterminal type III procollagen peptide (PIIINP), aminoterminal and carboxyterminal type I procollagen peptides (PINP and PICP) and cross-linked carboxyterminal telopeptide of collagen I (ICTP) were determined by radioimmunoassay (Orion Diagnostica, Espoo, Finland).

Capillary abnormalities were analysed quantitatively by a computer-based method [12]. The pulmonary artery pressure (PAPsyst) was measured in 30/48 patients by Doppler cardiology, which is a non-invasive technique allowing the pressure to be calculated from the velocity of the regurgitant flow through the tricuspid valve [13]. In 1/30 patients, there was no regurgitant flow and the pulmonary pressure could not be determined. Glomerular filtration rate (GFR) was determined by iohexol clearance [14] and expressed as the age-adjusted percentage of mean values for healthy controls. Pulmonary function was assessed as vital capacity (VC), measured by a dry spirometer, and as carbon monoxide diffusing capacity (DLCO), measured by the single-breath method; both were expressed as a percent-age. Skin involvement was assessed with a modified Rodnan score determined by standardized palpation of skin areas [15]. It has a theoretical range of 0–72.

Statistics

The significance of differences between patients and controls was calculated using the Mann–Whitney test for unpaired samples and correlation between pairs of parameters with Spearman’s ρ.

The serum ICTP concentration was increased both in dcSSc and in lcSSc compared with healthy controls (Table 1). Patients with dcSSc had higher levels than patients with lcSSc (P < 0.001). No difference in either of the collagen metabolites was found between smoking and non-smoking patients.
between serum PINP and ICTP or between serum PINP and PIINP concentrations.

Serum PIINP and ICTP concentrations both correlated positively with skin score (Table 2) and negatively with DLCO, but showed no correlation with kidney function, pulmonary pressure or disease duration. A correlation with the acute-phase reactant orosomucoid was found for serum PIINP and ICTP concentrations.

Discussion

Increased serum concentrations of PIINP, reflecting increased collagen III production, have been reported previously in SSc in several studies [7, 8, 16], whereas the serum PICP concentration has been found to be normal [9, 16]. In the present study we wanted to investigate both the synthesis and the degradation of collagen I and III. Patients with suspected SSc not fulfilling the ACR criteria were included as such patients often go on to develop SSc. Eight of 12 fulfilled the ACR criteria 2 yr after the initial assessment.

Collagen catabolism has been considered previously to be normal in SSc [4, 17]. However, Stone et al. [6] found increased urinary amounts of cross-linked amino acids specific for the degradation of mature collagen and elastin, with elevated levels of both hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP). As LP is regarded as originating mainly from bone, they suggested resorption of bone in the digits to be the explanation. They also found an increased HP/LP ratio compared with normal, indicating an origin of the HP in a soft tissue. The finding in the present study of an increased serum ICTP concentration in SSc suggests augmented collagen I breakdown, but the tissue origin of the metabolite is unclear. The majority of collagen I in the body is present in bone (50–70%), and as bone is a metabolically more active tissue than soft tissue, ICTP has been regarded as a marker preferentially reflecting bone collagen degradation [18]. Elevated serum levels of ICTP have been reported in rheumatoid arthritis, in which it is claimed to be an earlier marker of tissue destruction than radiography [19]. Patients with SSc are not very prone to the development of osteoporosis, especially not early in the disease. No patient had erosive joint disease in the present study and only 4/48 had acrolysis on X-ray (not shown). The ICTP levels of these four patients were 6.0, 4.5, 3.0 and 3.5 µg/l respectively. Furthermore, the bone-specific marker (BSP) was analysed in the dcSSc group with the highest ICTP levels, and normal values were found (not shown). Increased serum levels of ICTP have also been reported in Crohn’s disease [20] and in systemic lupus erythematous [21]. In none of these studies did the patients show signs of elevated bone resorption. Garnero et al. [22] found no difference between premenopausal women and postmenopausal osteoporotic patients in the serum concentration of ICTP, in contrast to other bone resorption markers, and no effect on ICTP levels of antiresorptive therapy with alendronate. Taken together with the observations reported in the present study, this suggests that the antigens measured in the ICTP assay are derived from tissues other than bone affected by SSc.

Heickendorff et al. [16] reported that the serum concentrations of PICP and ICTP covaried in systemic sclerosis. Their data showed increased ICTP levels in SSc patients but they did not report the ICTP values separately for the different forms of SSc. Similarly, Autio et al. [23] reported a correlation between the serum concentrations of PICP and ICTP, suggesting balanced synthesis and degradation in patients with different skin diseases, such as eczema, psoriasis and tinea. They also reported a low but significant correlation between PICP and PIINP concentrations, indicating coordinated synthesis of collagen I and III in these skin diseases. Such correlations were also noted in the present study. The highly significant correlation between ICTP and PIINP concentrations is more difficult to explain, but could indicate that the increased PIINP concentration also reflects degradation of tissue type III Pn-collagen, as has been reported after streptokinase treatment [24, J. Risteli, personal communication].

Wenisch et al. [25] reported a marked increase in the serum concentration ICTP lasting more than 4 weeks in gram-negative septicaemia. They suggested that this could be explained by an alteration of the extracellular matrix during vascular inflammation related to septicaemia. Since vascular inflammation is known to occur early in systemic sclerosis, this is an interesting hypothesis. Our results are supported by a recent paper by Hunzelmann et al. [26]. These authors reported an increased circulating ICTP concentration in SSc patients and suggested that the ICTP concentration is a marker of clinical severity, since the ICTP level correlated with the extent of the skin fibrosis. In the present study, the serum concentration of ICTP was found to correlate with skin score but also with acute phase reactants, and negatively with pulmonary function, which further underscores its potency as a marker of disease severity. Further studies may explore the tissue origin of the increased ICTP levels in SSc and the clinical usefulness of this metabolite.

Table 2. Correlation coefficient (r) between biochemical and clinical parameters in 48 patients with systemic sclerosis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Serum PICP</th>
<th>Serum PIINP</th>
<th>Serum ICTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin score</td>
<td>0.54**</td>
<td>0.14</td>
<td>0.32</td>
</tr>
<tr>
<td>DLCO</td>
<td>−0.46**</td>
<td>−0.12</td>
<td>−0.12</td>
</tr>
<tr>
<td>VC</td>
<td>0.20</td>
<td>0.32*</td>
<td>0.09</td>
</tr>
<tr>
<td>GFR</td>
<td>0.10</td>
<td>0.05</td>
<td>−0.24</td>
</tr>
<tr>
<td>PAPSyst</td>
<td>−0.14</td>
<td>−0.34</td>
<td>0.01</td>
</tr>
<tr>
<td>Capillary density</td>
<td>−0.42**</td>
<td>0.17</td>
<td>0.00</td>
</tr>
<tr>
<td>Disease duration</td>
<td>−0.22</td>
<td>0.09</td>
<td>0.13</td>
</tr>
<tr>
<td>CRP</td>
<td>0.27</td>
<td>0.08</td>
<td>0.11</td>
</tr>
<tr>
<td>Orosomucoid</td>
<td>0.38**</td>
<td>−0.03</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Significance of the correlation: *P < 0.05; **P < 0.01.

ESR = erythrocyte sedimentation rate.
Acknowledgements

This work was supported by grants from the Österlund Foundation, the Koch Foundation, The Medical Faculty of the University of Lund, and the Medical Research Council (project no K97–19X-11628–02B).

References


