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Increased Collagen Propeptides in the Skin of a Scleredema Patient but No Change in Re-epithelialisation Rate

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Scleredema is a rare disease, affecting the skin connective tissue with increased amounts of collagen and glycosaminoglycans. In the present study, the collagen synthesis and re-epithelialisation rate were measured from a 64-year-old male patient, who rapidly developed extensive tightening of the skin, without any underlying disease. The skin was thickened at several sites when measured with ultrasound, and the histology revealed accumulation of glycosaminoglycans and collagen bundles. The collagen synthesis rate was measured from suction blisters induced on two different sites of the skin before the treatment and three times later up to 6 months after the treatment with a systemic steroid was started. The aminoterminal propeptide of type I collagen (PINP) was increased manifold in the affected skin when compared with the controls, indicating active collagen deposition in vivo. Systemic steroid medication with high doses (over 20 mg/d) decreased both the type I and the type III collagen propeptide levels. The time schedule of the decreases in the propeptides in the thickened, affected skin and in the clinically normal-looking skin varied, and especially in the thickened skin in the abdomen the decrease in PINP was noted only after 3 months of prednisolone therapy. When the prednisolone dose was only 10 mg daily, the propeptides were again up-regulated, perhaps reflecting the natural course of the disease.

The re-epithelialisation rates at two different sites of the patient were similar to those in the controls, suggesting that even massive fibrosis with active deposition of collagen does not alter the basal rate of re-epithelialisation in the skin.

In conclusion, collagen synthesis is markedly elevated in scleredema, leading to fibrosis of the skin. A recently developed method utilizing assays of collagen propeptides from suction blister fluid allows monitoring of the collagen synthesis and detection of changes in the collagen synthesis during the treatment of fibrotic disorders.

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Scleredema is a rare connective tissue disease, characterized by thickening and induration of the skin. It may be associated with diabetes or paraproteinemia, or it may develop after some febrile disease (1–4).

Studies have revealed increased amounts of both glycosaminoglycans and collagen in scleredema skin. Increased synthesis of collagen, as demonstrated by cell culture studies, and elevated collagen mRNA levels have been suggested to lead to the thickening of the skin (5, 6).

In the present study, we investigated the collagen synthesis rate with a newly developed method in a patient who had scleredema with rapid onset. Furthermore, we followed the collagen synthesis rate during the course of the disease while the patient was treated with systemic steroid medication.

PATIENT AND METHODS

Patient

The patient was a 64-year-old male who rapidly developed skin thickening involving the upper arms, the chest, the abdomen and the back and later on the thighs (Fig. 1). During the thickening of the skin, there was no pain in the joints or dysphagia. Due to skin symptoms a systemic prednisolone treatment was started from a dose of 40 mg daily (see also Fig. 3).

In 1980 and 1985 the patient had had a heart attack, and in 1992 a heart bypass operation had been made, in which six vessels had been replaced. The patient also has claudication due to severe atherosclerosis and regularly takes nifedipine 20 mg daily and acetylsalicylic acid 250 mg daily. Laboratory investigations revealed the following values: serum (S) follicle stimulating hormone and luteinizing hormone were slightly elevated, being 15.2 (<10 U/l) and 13.9 (1–9 U/l), respectively. S-prolactin was 8.3 (<15 µg/l) and S-insulin 7.7 (2.4–20.2 mU/l). S-calcium, S-phosphate and blood glucose were normal. Serum alkaline phosphatase was slightly elevated: 430 (60–250 U/l) and isoenzyme analysis showed it to be from the liver.

Serum cortisol was 0.11 and 0.57 (0.15–0.65 µmol/l). Serum growth hormone was 0.25 (<7 µg/l) and creatinine kinase 36 (<280 U/l). Serum thyreoglobulin antibody was <25 (<25).

Serum porphyrines and electrophoresis were normal, without any evidence of paraproteinemia. Antinuclear, anticentromere and anti-scleroderma-70 autoantibodies were negative. There were no abnormalities in the thorax and sella X-rays. Bone marrow was also normal.

Histology, immunohistochemistry and electron microscopy

Skin samples were obtained under local anaesthesia from the thickened skin of the upper arm and the chest. For histological and immunohisto-

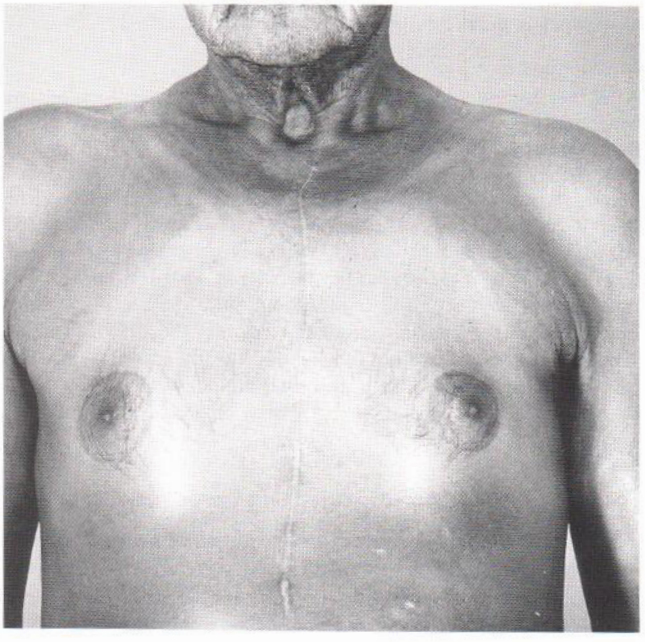

Fig. 1. Clinical picture of the scleredema patient.

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chemical stainings, the excised specimens were fixed in 10% buffered formalin, embedded in paraffin and cut into 4-μm sections. Hematoxylin-eosin, Alcian blue-PAS and Verhoeff stainings were performed. The sections for immunohistochemistry were pretreated with trypsin digestion (for anti-elastin) or pepsin digestion (for anti-PINP and PIIINP) and by microwave oven heating (for anti-PINP). The immunohistochemical stainings were performed with the avidin-biotin method using an ABC-HRP kit (Dako A/S, Glostrup, Denmark) according to the manufacturer's instructions. Diaminobenzidine was used as a chromogen and, for the negative controls, the primary antibodies were substituted by the buffer solution. The antibodies for the procollagens PINP and PIIINP were from Drs Leila Risteli and Juha Risteli (Department of Clinical Chemistry, University of Oulu, Oulu), and anti-elastin was a commercial antibody (Clone-BA 4, SigmaImmunoChemicals Co, St. Louis, USA).

The specimens for electron microscopy from the patient were fixed in 4% glutaraldehyde, post-fixed in 1% osmium tetroxide and embedded in epon. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under a Philips 410 LS transmission electron microscope.

Measurement of skin thickness

Skin thickness was measured ultrasonically using a Dermascan A (Cortex Technology, Denmark) at four locations: the abdomen, the lateral aspect of the upper arm and the forearm, the dorsal aspect of the foot and the soft-looking skin on the upper chest. The measurements were made three or four times at each time point in all the locations. The values are expressed as the means.

Measurement of collagen propeptides

At each time point, five blisters were induced at two different sites: on the upper chest in the area of soft skin and on the abdomen in the markedly indurated skin. The suction blisters were induced according to the method of Kiistala (7), using a Dermovac disposable suction device (Ventipress, Lappeenranta, Finland) with a negative pressure of 180–240 mmHg. The blister fluid was collected and stored at −20°C. From the blister fluid, PINP and PIIINP were measured using radioimmunoassays with commercial reagent kits (8–10). The PINP and PIIINP concentrations were assayed using a sequential saturation method to increase the sensitivity of the assay (8). Propeptides were assayed from 11 controls (males, mean age 64 years). Serum samples for carboxyterminal propeptide of type I collagen (PICP) and PIIINP and the collagen degradation marker, ICTP, were assayed using radioimmunoassays (9, 11, 12).

Measurement of re-epithelialisation rate

After the blister fluid had been collected, the epidermis of the blisters was removed. Water evaporisation was measured from every blister floor wound as transepidermal water loss (TEWL), (Evaporimeter EP1, Servomed, Sweden) (13, 14). The measurements were made immediately after the induction of the blisters and after 4 days. The re-epithelialisation rate was also studied in 11 controls (mean age 52 years), in whom the measurements were similarly made after 2 days. Our studies have revealed that the decrease of TEWL is linear for at least up to 4 days after blister induction. Since there is slight water evaporisation even in skin with intact epidermis, the TEWL of intact skin was also measured in all cases, and this value was subtracted from the values obtained from the blister floors.

RESULTS

Histology, immunohistochemistry and electron microscopy

The dermal collagen bundles throughout the thickened reticular dermis were large and intimately surrounded the sweat glands and hair follicles. Small lymphocytic infiltrates were seen around some blood vessels, and small amounts of Alcian blue-positive acid mucopolysaccharides were found between the collagen bundles, especially in the upper dermis and, to some extent, in the lower dermis. Verhoeff staining for elastic tissue showed normal-looking elastic fibres, but their relative amount seemed to be diminished, apparently because of the increase of collagen.

Immunohistochemical stainings for the procollagens PINP and PIIINP revealed positively stained fibrils in basement membrane areas, both subepidermally and around hair follicles and sweat glands. The thickened dermal collagen bundles also showed some staining for PIIINP. Anti-elastin showed a finding similar to the Verhoeff staining, and the relative amount of elastin fibrils seemed to be decreased.

The electron microscopic study of the scleredema skin showed some increase of active-looking dermal fibroblasts with a prominent rough endoplasmic reticulum. Regular collagen fibrils were surrounded by accumulation of some amorphic material, consistent with proteoglycans, and some fibrillar material with very short and thin fibrils (Fig. 2). The elastic fibres were normal-looking or showed minor irregularities in the shape of the borders.

Skin thickness

The skin was thickened at several sites compared to the controls (Table I). The thickness of the soft skin in the upper chest was 2.0 mm, which was within the normal range.

Levels of collagen propeptides

The collagen propeptides PINP and PIIINP were measured from the abdominal (tight, indurated skin) and the chest (soft skin with normal consistency) skin using the suction blister method. Before the systemic treatment was started, the level of PINP was over two-fold compared with the mean +1 SD of the age-matched controls (Fig. 3). In the upper chest skin

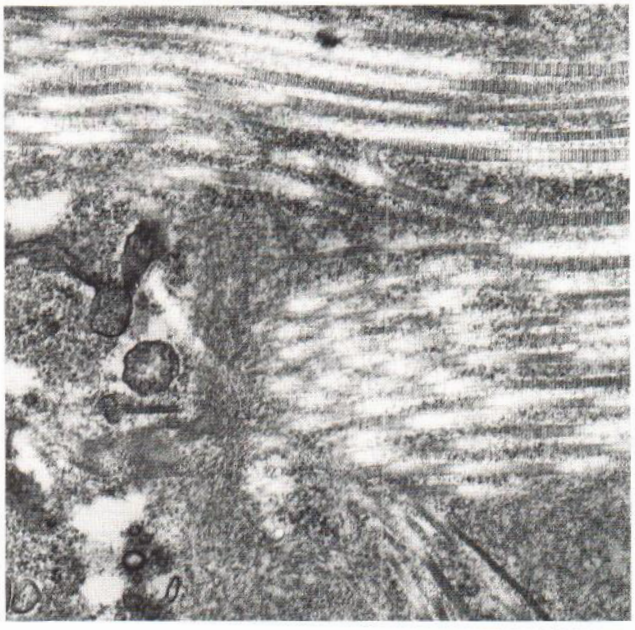

Fig. 2. An electron micrograph of the scleredema skin shows regular collagen fibrils at the top of the figure and accumulation of finely granular and fibrillar material with very short and thin fibrils at the center and bottom of figure. Magnification ×24,000.

Table I. *The thickness of the skin of the scleredema patient*
The values are the mean of three or four measurements (mm).

Site	Date				Controls ($n=9$)
	8.2	21.3	8.5	14.8	
Abdomen	3.19	2.64	2.41	2.51	1.88 ± 0.21
Chest	2.90	2.98	2.70	2.81	ND
Upper arm	3.21	2.64	2.46	2.53	1.68 ± 0.23
Lower arm	3.09	2.63	2.64	2.27	ND

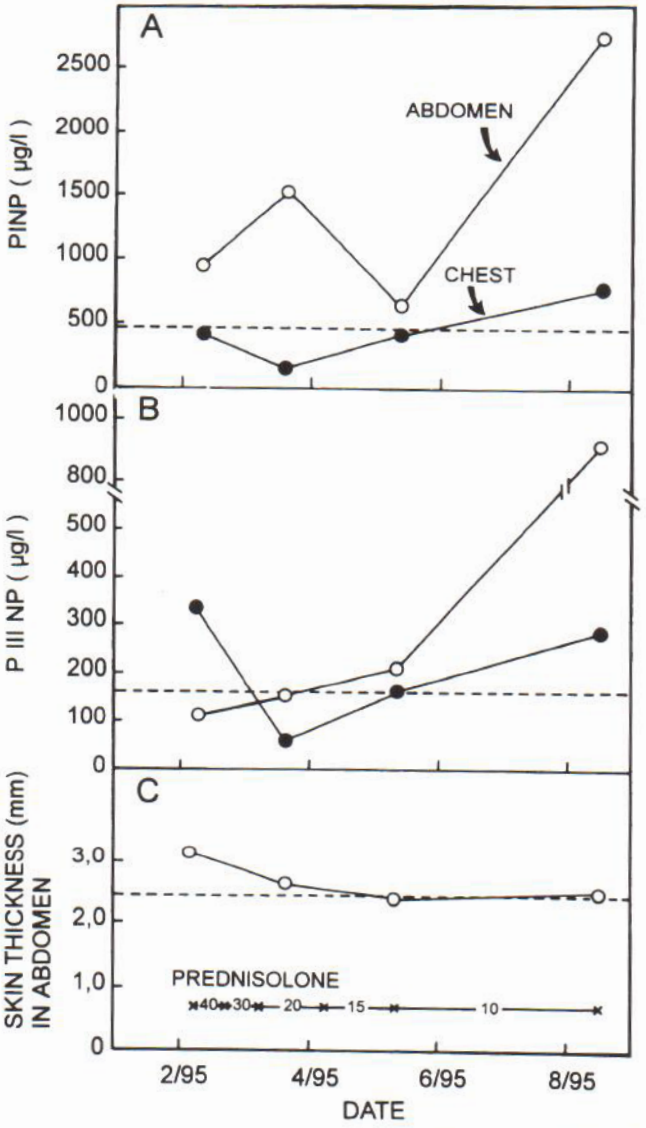

Fig. 3. The levels of the collagen propeptides PINP (A) and PIIINP (B) and skin thickness (C) in the affected, tight abdominal skin of the scleredema patient and the levels of propeptides in the healthy-looking, soft skin of the upper chest. Suction blisters were induced as described on the abdominal and chest skin at different time points, and the collected blister fluids were analysed for PINP and PIIINP. The dotted line indicates the mean + 1 SD of healthy age-matched controls. Skin thickness was measured from the abdominal skin by ultrasound (Dermascan A) at different time points. The dotted line indicates the mean + 1 SD of the controls. abdomen (○—○), chest (●—●).

Table II. *The levels of collagen propeptides PINP and PIIINP and crosslinked telopeptide of type I collagen (ICTP) in the serum of the scleredema patient*

Reference range	Date			
	9.2	21.3	8.5	14.8
PICP (38—202 µg l)	91	98	106	127
PIIINP (1.7–4.2 µg l)	3.9	4.0	3.6	3.9
ICTP (1.6–4.6 µg l)	ND[a]	8.4	7.2	7.6

[a] ND = not documented.

the PINP values were within the normal range, and after the introduction of the systemic steroid there was a marked decrease in the PINP value in this area. The PINP value in the abdominal skin, however, continued to increase although the steroid dose was relatively high, but there was a decrease in PINP 3 months after the beginning of the treatment. Surprisingly, at 6 months the PINP value of the abdominal skin was markedly increased when the dose of prednisolone was 10 mg daily. The values of PIIINP behaved differently. Before the beginning of the steroid treatment, PIIINP was within the normal range in the abdominal skin. Especially the chest skin showed a marked decrease in PIIINP when the dose of prednisolone was high (over 20 mg/d). Six months after the beginning of the treatment, the PIIINP value was markedly increased, especially in the abdominal skin.

In the serum, neither PICP nor PIIINP was increased before the treatment or during the follow-up when compared to the reference range. The collagen degradation marker, ICTP, was constantly slightly higher than the corresponding reference values (Table II).

Re-epithelialisation rate

The re-epithelialisation rate of the suction blisters was measured before the systemic steroid treatment. In the intact skin of the patient's chest (soft skin), TEWL was 5 g/m^{2}h (mean of six determinations) and in the abdomen (tight skin) 7 g/ m^{2}h. These values are close to the values obtained in the 11 controls (mean value 4.4 ± 2.9 g/m^{2}h).

After the blister roofs were removed, TEWL was 113 g/m^{2}h on the blisters induced on the chest, and 104 g/m^{2}h on the abdomen. (The baseline TEWL of intact skin had been subtracted from the measured values). There was a marked decrease in TEWL during 4 days, and the values were 43 g/m^{2}h and 38 g/m^{2}h in the chest and the abdomen. As can be seen, these values were within the range of the controls, and the slope of decrease of TEWL was similar in the patient and in the controls (Fig. 4).

DISCUSSION

The recently developed methodology used in our study clearly demonstrated the increased collagen synthesis rate in the affected skin of a scleredema patient. The finding is consistent with our previous studies, where cell cultures were used (5); a recent study utilizing the in situ hybridization technique revealed active fibroblasts containing abundant collagen mRNA to be present in the skin of a scleredema patient (6).

In our present study, the collagen synthesis rate was followed

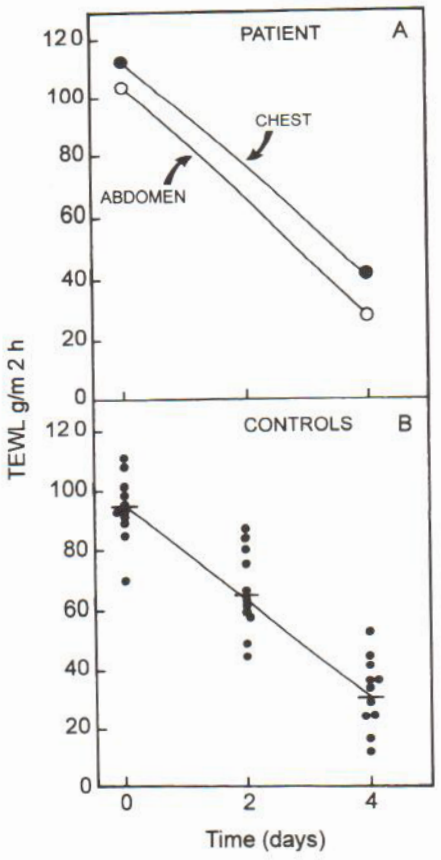

Fig. 4. The decrease of transepidermal water loss (TEWL) in a scleredema patient and controls during re-epithelialisation. TEWL was measured immediately after blister induction from the blister bases after removal of the blister roofs and 4 days later. The measurements in the controls were also made 2 days after blister induction. As can be seen, there was a linear decrease in TEWL for up to 4 days (as shown in the controls). The slope of the decrease in TEWL in the abdomen and the chest was similar to that in the controls. The values are the means of five blister bases, each measured in triplicate.

by measuring the collagen propeptides in the blister fluid. During collagen synthesis, propeptides are cleaved off from the procollagen and the amount of propeptides thus reflects the actual ongoing collagen synthesis (8). Our previous studies have shown that this method is highly useful when, for example, the effect of systemic or topical glukocorticoids on the collagen synthesis is studied and, further, that the method is sensitive enough to detect relatively small changes in collagen synthesis (8, 15). Recently, Heichendorff et al. reported increased levels of collagen propeptides in suction blister fluid induced into lesional skin of systemic scleroderma patients (16). However, our study is the first in which collagen propeptides were measured in a scleredema patient and, furthermore, the effect of a systemic steroid was studied. Indeed, in the present study we were able to demonstrate that a high dose of prednisolone, over 20 mg daily, decreased the collagen propeptides PINP and PIIINP in soft, healthy-looking skin, which is in agreement with a recent paper by Autio et al. (15). The decrease in the affected skin took place later. However, when the dose of prednisolone was 10 mg daily, the propeptides were again elevated, suggesting that the collagen synthesis rate was again high. This supports the finding of Varga et al. that collagen synthesis may be activated in scleredema patients for a relatively long time, i.e. up to several years (6). Surprisingly, the degradation product of type I collagen, ICTP, was constantly elevated in the serum of the scleredema patient. ICTP is mostly derived from bones (12), but some of it may also come from the skin. It is possible that scleredema also involves an increase in collagen degradation, as it has been observed in patients with systemic scleroderma (16). In contrast, the levels of PICP and PIIINP in the serum of the patient were not increased, which is in agreement with a previous study in which an increase in serum levels of PIIINP was not observed in scleredema patients (17).

In the present study, re-epithelialisation of fibrotic skin was also studied. The method used is based on the reduction of TEWL in blister bases. The epithelialisation rate was similar both in the healthy-looking chest skin and in the tight, affected skin of the abdomen, and it was not altered in comparison to the controls. This suggests that the re-epithelialisation rate does not correlate with the rate of type I collagen synthesis and that even in highly fibrotic skin the re-epithelialisation reflecting keratinocyte migration and differentiation is intact.

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