Collagen Metabolites in Urine in Localized Scleroderma (Morphoea)

THORKIL AMMITZBÖLL and JØRGEN SERUP

Department of Dermatology (with Connective Tissue Research Laboratories), Rigshospitalet, Copenhagen, Denmark


Hydroxyproline (Hyp), hydroxylysine (Hyl), and proline (Pro) were assayed in the urine from 28 patients with localized scleroderma (13 with localized morphoea plaques, 13 with generalized morphoea, and two with scleroderma en coup de sabre), as well as 17 control persons. The levels of Hyp, Hyl and Pro were elevated in patients with localized morphoea plaques as compared to the controls indicating major changes of the collagen metabolism in this type of localized scleroderma. Key words: Hydroxyproline; Hydroxylysine; Proline; Collagen; Urinary; Circumscribed. (Received June 28, 1984.)

Localized scleroderma is considered a local skin disease with circumscribed lesions of scleroderma in contrast to normal appearing skin. The plaques or lines of localized scleroderma are characterized by excessive collagen deposition in the dermis. Internal organs are assumed not to be involved.

Generalized scleroderma (progressive systemic sclerosis) differs from localized scleroderma in being a systemic disorder of connective tissue with fibrotic changes of the skin, the gastrointestinal tract, the lungs, the heart and vessel walls, and a variety of other internal organs. The altered collagen metabolism in generalized scleroderma is reflected in an increased urinary excretion of hydroxyproline and hydroxylysine, i.e. amino acids characteristic of collagen (1, 2).

In this study, the urinary excretions of hydroxyproline (Hyp), hydroxylysine (Hyl) and proline (Pro) by patients with localized scleroderma were assayed. To the best of our knowledge this study is the first on urinary excretion of metabolites of collagen in localized scleroderma.

MATERIAL AND METHODS

Patients

The study included 28 patients with a clinical and light-microscopical diagnosis of localized scleroderma, i.e. 13 patients (11 women, 2 men) with localized morphoea plaques (LMP), 13 patients (all women) with generalized morphoea (GM), and 2 patients with scleroderma en coup de sabre (CS). Patients with LMP presented a few sclerotic plaques of morphoea in a few regions, while patients with GM presented several plaques in several regions. Mean age of patients with LMP was 32.9 years (16-48), of patients with GM 56.6 years (33-75), while the two patients with CS were 18 and 51 years old. Mean duration of localized scleroderma was 6.7 years (0.5-19) in patients with LMP, 3.1 years (0.3-8) in patients with GM, while the duration was 4.1 and 0.8 years, respectively, in the two patients with CS. Clinical signs of activity of the disease, i.e. inflammation with a lilac ring or larger extension of the circumscribed lesions of scleroderma for the previous 3 months were noted in 10 patients with LMP and in 10 patients with GM, while none of the patients with CS exhibited signs of activity. None of the patients received systemic treatment, and no patient was treated locally in relation to the study.

For control, 17 persons (16 women, 1 man) with a mean age of 52.5 years (26-71) were studied. Five were healthy, 4 had various localized skin diseases (dermatophytosis, keratodermia palmo-plantaris, condyloma acuminatum) and 8 had generalized skin diseases (psoriasis, atopic dermatitis, senile pruritus, urticaria pigmentosa). Statistical analysis for metabolites of collagen in the urine showed that
Acta Derm Venereol (Stockh) 64

**Short reports 535**

**Fig. 1.** Urinary excretion of total hydroxyproline in patients with localized morpoea plaques (LMP), generalized morpoea (GM), and in control persons.

**Fig. 2.** Urinary excretion of polypeptide-bound hydroxyproline in patients with localized morpoea plaques (LMP), generalized morpoea (GM), and in control persons.

There were no significant differences between the subgroups of controls, and no special tendencies were found. Furthermore, no correlation to age was found (correlation coefficients were for Hyp total \(0.06\), Hyp polypeptide \(-0.27\), Hyl 0.15, Pro total \(-0.28\), Pro polypeptide \(-0.01\)). None of the patients or controls had proteinuria or evidence of renal disease.

**Collection of 24-hour urine**

All persons received a collagen free diet for 24 hours prior to and during collection of a 24-hour urine sample. The urine was filtered and kept at \(-20^\circ \text{C}\) until analyzed.

**Table I. Urinary excretion of collagen metabolites in localized scleroderma**

The urinary excretion of Hyp, Hyl and Pro in micromol/24 h are given as mean ± SEM and 10-90 percentiles.

<table>
<thead>
<tr>
<th></th>
<th>Total urinary excretion</th>
<th>Polypeptide-bound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hyp</td>
<td>Pro</td>
</tr>
<tr>
<td>Localized morphoea plaques</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>384±51 (214-560)</td>
<td>505±63 (352-650)</td>
</tr>
<tr>
<td>Generalized morphoea</td>
<td>272±24 (186-410)</td>
<td>426±18 (359-547)</td>
</tr>
<tr>
<td>Scleroderma en coup de sabre</td>
<td>825, 335</td>
<td>682, 450</td>
</tr>
<tr>
<td>Controls</td>
<td>248±14 (185-322)</td>
<td>358±16 (277-434)</td>
</tr>
</tbody>
</table>
Analyses

Hyp and Hyl are characteristic constituents of collagen, and Pro is a major constituent. However, Pro occurs in many other proteins as well. The total urinary excretion of Hyp and Pro and the urinary polypeptide-bound Hyl, Hyp and Pro (1+5 fraction) were measured by an automated colorimetric procedure (3, 4). The results were expressed as micromol/24 h. The limits of detection were 5 micromol Hyp/24 h, 2 micromol Hyl/24 h, and 10 micromol Pro/24 h. The reproducibility, i.e. the variation of double estimations of Hyp, Hyl and Pro were 1.3%, 5% and 1.5%, respectively.

Urinary polypeptide-bound Hyp, Hyl and Pro. A mixture of 1.0 ml urine and 5.0 ml acetone was kept at 0°C for one hour and centrifuged at 800 g for 15 min. The precipitate was hydrolysed by 2.0 ml 6 N HCl in a sealed ampoule at 118°C for 18 hours. The hydrolysate was evaporated to dryness at 60°C at 50 mbar over solid NaOH, reconstituted in 4 ml of buffer and analysed for Hyp, Hyl and Pro in an AutoAnalyzer® equipment.

Total urinary Hyp and Pro. Urine (1.0 ml) was mixed with 1.0 ml 12 N HCl, hydrolysed, evaporated and analysed for Hyp and Pro.

Statistical evaluation

The Wilcoxon rank sum test for unpaired data and the variance ratio test (F-test) were used for the statistical analyses. Biological significance was recognized at p<0.05.

RESULTS

The total urinary excretion of Hyp and Pro as well as the urinary excretion of polypeptide-bound Hyp, Hyl and Pro are shown in Table I and Figs. 1-2.

The variances of all the parameters analysed, except for the excretion of Hyl, were significantly higher in LMP than in the controls (F-test).

Total Hyp, total Pro and polypeptide-bound Hyp were significantly increased in LMP as compared to the controls. Total Pro and polypeptide-bound Pro were increased in patients with GM as compared to the controls. Elevated values of both total Hyp and polypeptide-bound Hyp, as defined as values higher than the range of the controls, were found in four of the 13 patients with LMP, one of the 13 patients with GM and one of the two patients with CS. These patients did not differ from the other patients with respect to clinical data. Ages of patients with elevated urinary Hyp, both total and polypeptide-bound, were 15, 18, 27, 31, 35, and 47 years. Differences between patients with LMP and controls, and between patients with GM and controls were still significant, if the two younger patients were excluded.

Patients with localized scleroderma did not differ from the controls in respect to the ratio of polypeptide-bound Hyp to total Hyp. In generalized scleroderma this ratio is consistently increased (1, 2).

DISCUSSION

The excretion of urinary metabolites of collagen was raised in the group of patients with LMP, and so was the variance of the excretions as compared to the controls. Some of the patients with LMP had a normal excretion, while it was increased in others, the latter finding indicating major changes in the collagen turnover. It is of interest to notice that some patients with LMP and clinically minor skin involvement had abnormal urinary excretion of metabolites of collagen in contrast to patients with GM, in which larger areas of skin is clinically involved. Recently, it was reported from this department that patients with LMP have thickening of the cornea but no other evidence of specific eye affection (5). The cornea is composed of an avascular connective tissue stroma and a thin epithelium and endothelium. Increased cornea thickness in patients with LMP probably reflects changes in the composition of the glycosaminoglycans of the cornea (5). The cornea thickness is also increased in generalized scleroderma (6). Thus, the finding of increased
urinary excretion of metabolites of collagen in the present study provides further evidence that morphea, preferably LMP, is not simply a local alteration of connective tissue confined to the plaques, but a more generalized biochemical abnormality of connective tissues.

ACKNOWLEDGEMENTS
The authors are greatly indebted to Mr Ole Christensen, Mrs Grethe Krömer and Mrs Shinko Hammen for expert technical assistance, and to Mrs Inger Johansson for her typing the manuscript.

REFERENCES

Measurement of Transepidermal Water Loss Using an Evaporimeter to Follow the Restitution of the Barrier Layer of Human Epidermis after Stripping the Stratum corneum
THOMAS FRÖDIN and MARCUS SKOGH
Department of Dermatology, University Hospital, Linköping, Sweden


The rate of evaporation after stripping a test area with adhesive tape was followed in 10 women over a period of 15 days. The much increased transepidermal water loss after stripping diminished rapidly on the first 3 days. It then decreased more slowly, approaching but not fully reaching the normal rate by the end of test period. This can be taken to reflect the repair process of the damaged barrier layer. The instrument used makes possible instantaneous reading of the rate of evaporation, and is highly suited for assessing the function of the barrier layer. (Received July 4, 1984.)

T Frödin, Department of Dermatology, University Hospital, S-581 85 Linköping, Sweden.

The stratum corneum acts as a barrier for water (1). By damaging this barrier, for example by stripping the skin with adhesive tape, the water loss increases greatly (2, 3, 4). Measurement of water loss could therefore be used for assessing the function of the barrier layer.

Evaporation from normal skin comprises sweating and transepidermal diffusion. Envi-