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Collagen Synthesis in Skin Tissue from Patients with Systemic Scleroderma

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ABSTRACT. Synthesis of total and collagenous protein in systemic scleroderma skin tissue was determined. Biopsy specimens of forearm and abdominal skin from 7 patients with scleroderma and 3 normal subjects were used. The total protein synthesized was measured by the incorporation of radioactive proline using the tissue, and the collagenous protein synthesized was measured by the incorporation of labeled hydroxyproline. The ratio of forearm skin to abdominal skin in scleroderma on total and collagenous protein synthesis per protein was lower than in normal controls. Total and collagenous protein synthesis per protein of forearm skin taken from the scleroderma patients tended to show lower values than those obtained from the normal controls. It was indicated that collagen synthesis per protein decreased in skin tissue from the sclerotic area in scleroderma.

Key words : Systemic sclerosis — Synthesized collagen — Sclerotic area

Systemic scleroderma, a generalized disorder of the connective tissue, causes fibrosis of the skin and various organs. Therefore, many investigations of scleroderma from the aspect of collagen metabolism, particularly of collagen synthesis, have been done. Most of these studies using cultured skin fibroblasts indicate that fibroblasts from patients with scleroderma increase collagen synthesis as compared with those from normal controls.[1—4] However, investigation of the collagen synthesis of sclerodermal skin tissue by the incorporation of labeled precursors to skin tissue has not been carried out, excepting one earlier preliminary study.[5] In scleroderma, skin of the forearm is apt to be frequently affected, while abdominal skin is rarely affected.[6] In this study, we report on collagen synthesis by means of the incorporation of labeled proline employing an organ culture of forearm and abdominal skin biopsied at the same time and comparison with that of normal human skin.

MATERIALS AND METHODS

Sclerodermal skin samples

Seven patients who were clinically and histopathologically diagnosed as having systemic scleroderma were selected for this study. Some of the clinical data on these patients is presented in Table 1. None of the patients were on systemic therapy. All biopsies of systemic scleroderma skin were taken from

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TABLE 1. Clinical data of scleroderma patients used in the study.

Case No.	Age, sex	Duration of disease	Stage	Sclerosis forearm	Sclerosis abdomen	Clinical picture
1	30, f	1 yr	Sclerotic	+++	−	Sclerodactyly Raynaud's phenomenon
2	33, f	2 yr	Sclerotic	+++	−	Sclerodactyly digital pitting scar skin ulcers Raynaud's phenomenon
3	47, f	2 yr	Sclerotic	+++	+	Sclerodactyly Raynaud's phenomenon pulmonary fibrosis
4	48, f	3 yr	Sclerotic	++	−	Sclerodactyly skin ulcers Raynaud's phenomenon pulmonary fibrosis
5	54, f	12 yr	Sclerotic	+	−	Sclerodactyly skin ulcers Raynaud's phenomenon pulmonary fibrosis
6	58, f	6 yr	Sclerotic	+	−	Sclerodactyly Raynaud's phenomenon pulmonary fibrosis
7	58, m	5 yr	Sclerotic	+	−	Sclerodactyly digital pitting scar Raynaud's phenomenon

+=mild, ++=intermediate, +++=prominent

the forearm and abdomen. Control samples were taken from the forearm and abdomen of 3 patients during plastic surgery.

Labeling of skin tissue

The entire dermis was immediately taken from the skin biopsy samples manually and was cut into slices of 1 mm. Then the materials (30–50 mg wet weight) were incubated for 3 hours at 37°C under 95% air : 5% CO_2 in 5 ml of Dulbecco's modified Eagle's Medium [Nissui Seiyaku Co., Tokyo, Japan] supplemented with 3.4 mg/L α-ketoglutarale Na salt, 50 mg/L ascorbic acid Na and 5 μCi/ml of L-[3,4-^{3}H] proline [spec. act. 39.6 Ci/mmol ; NEN Chemicals, Boston, Mass., USA].

Determination of incorporated radioactivity into total and collagenous protein

After incubation, the materials were harvested and washed with phosphate buffered saline. The materials were treated in 1.5 ml of 1 N NaOH and were stirred at room temperature for 24 hours. From each preparation, 0.5 ml sample was used for the assay of protein content. To the remaining sample, trichloroacetic acid (TCA) was added in a final concentration of 10%, and TCA insoluble precipitates were collected. The precipitates were hydrolyzed in a sealed Pyrex tube with 2 ml of 6 N HCl at 125–135°C for 6 hours. The hydrolysates were dried in vacuo and dissolved in 4 ml of distilled water. Aliquots of 0.5 ml were transferred into counting vials, mixed with 10 ml Aquasol [NEN Chemicals, Boston, Mass., USA], and then radioactivity was determined. The radioactivity was calculated as newly synthesized proteins. Labeled hydroxyproline was assayed from the remaining hydrolysate, according to the method of Juva and Prockop[7] and was estimated as the amount of

collagen sythesized. The percentage of total protein synthesized as collagen was calculated from the radioactivities in total protein synthesized and in collagenous protein synthesized, according to the assumption of Diegelmann and Peterkofsky.[8]

Measurement of protein

Protein concentration was measured according to the method of Lowry *et al.*,[9] using bovine serum albumin as a standard.

RESULTS

Table 2 presents data on the total and collagenous protein synthesis of sclerodermal and normal skin obtained from the forearm and abdomen. The ratio of forearm skin to abdominal skin on total and collagenous protein synthesis per protein was lower in scleroderma than in normal controls. Total and collagenous protein synthesis per protein of forearm skin taken from scleroderma patients tended to show lower values than those obtained from the normal controls. The percentage of collagen to the total protein synthesis of scleroderma and normal skin revealed a similar varying between 8.7 and 13.7% in all samples. However, in those scleroderma patients who had severe sclerotic changes on the forearm (Cases 1-4), the collagen percentage of forearm skin tended to be lower than that of abdominal skin.

TABLE 2. Total protein and collagen synthesis of sclerodermal and normal skin obtained from forearm and abdomen.

	Ratio of forearm to abdomen of total protein synthesis	Ratio of forearm to abdomen of collagen synthesis	% collagen in newly synthesized proteins	
Scleroderma			forearm	abdomen
1	1.48 (310, 209)	1.32 (90, 68)	11.6	13.0
2	1.19 (144, 121)	1.11 (40, 36)	11.2	12.0
3	2.64 (552, 209)	2.39 (165, 69)	12.0	13.2
4	2.02 (384, 190)	1.85 (120, 65)	12.5	13.7
5	2.18 (604, 277)	2.28 (161, 70)	10.6	10.2
6	1.69 (260, 154)	1.74 (58, 34)	8.9	8.7
7	2.58 (177, 69)	2.88 (51, 18)	11.5	10.3
Mean±SE	2.0±0.2(347±67,176±26)	1.9±0.2(98±20,51±8)	11.2±0.4	11.6±0.7
Normal controls				
1 (20,m)	3.30 (628, 190)	3.42 (199, 58)	12.7	12.3
2 (24,f)	3.06 (423, 138)	2.93 (106, 36)	10.0	10.5
3 (35,f)	3.69 (626, 170)	3.80 (209, 55)	13.4	12.9
Mean±SE	3.4±0.2(559±68,169±15)	3.4±0.3(171±33,50±7)	12.0±1.0	11.9±0.7

Total protein synthesis was measured by incorporation of ^{3}H-proline (forearm, abdomen [10^2 dpm/mg·protein]); collagen synthesis was measured by incorporation of ^{3}H-hydroxyproline (forearm, abdomen [10^2 dpm/mg·protein])

DISCUSSION

Many studies of collagen synthesis using skin fibroblast culture systems have been done. LeRoy[1] found an increase in collagen production in scleroderma

fibroblasts. This observation was later corroborated by a number of investigations.[2–4] On the other hand, Perlish *et al.*[10] reported that in fibroblasts isolated from sclerodermatous skin, all cell lines failed to show a significant increase in collagen synthesis as compared to control fibroblast lines.

As to methods for investigation of collagen synthesis in skin tissue in scleroderma, there are two. With the first method, determination of protocollagen proline hydroxylase (PPH) activity acts as an index of collagen synthesis. PPH levels have been reported to be variably elevated in skin biopsy materials from scleroderma patients.[11,12] The second method, which was employed in our study, is to measure the incorporation of the labeled precursor of collagen in skin tissue. In 1969, Keiser & Sjoerdsma[5] reported increased collagen synthesis in skin tissue obtained from several scleroderma patients in their preliminary study. The report, however, compared only one normal subject and 6 subjects of miscellaneous disorders such as lupus erythematosus and rheumatoid arthritis and did not investigate the same biopsy sites.

In this study, we investigated collagen synthesis by the incorporation of labeled precursors of forearm and abdominal skin. It was demonstrated that in scleroderma the ratio of forearm skin to abdominal skin on total and collagenous protein synthesis per protein was lower than in the normal controls. Considering that on the forearm collagen synthesis per protein in scleroderma tended to be lower than in the normal controls, and that in some cases with marked sclerosis the percentage of collagen to total protein in forearm skin tended to be smaller than in abdominal skin, it is suggested that collagen synthesis-involved skin in scleroderma decreases as compared with that in normal controls. The above-mentioned results do not agree with many studies which have investigated collagen synthesis by means of incorporation of cultured fibroblasts from scleroderma.

In the fibrotic stage large quantities of fibrous material are deposited and cause sclerosis of the affected organs.[13,14] A diffuse or perivascular inflammatory infiltration of the subcutaneous fat and of the cutis is observed in the initial stage of the disease.[15] Recently, many authors have reported that factors derived from lymphoid cells and macrophages play an important role in inflammation-stimulated fibroblast proliferation and collagen and noncollagen protein synthesis.[16–18] Therefore, we suppose that collagen synthesis increases in the early stage of scleroderma.

Our system using skin tissue to measure the incorporation of the labeled precursor of collagen which has enabled us to measure collagen synthesis in the presence of cell-matrix interaction and serum factors, appears to be superior to other methods using cultured skin fibroblasts as representative of the *in vivo* status. Our data show that collagen synthesis per protein decreases in skin tissue from sclerotic areas in systemic sclerosis.

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REFERENCES

1) LeRoy, E.C. : Increased collagen synthesis by scleroderma skin fibroblasts in vitro.

A possible defect in the regulation or activation of the scleroderma fibroblast. J. Clin. Invest. **54** : 880–889, 1974
2) Buckingham, R.B., Prince, R.K., Rodnan, G.P. and Taylor, F. : Increased collagen accumulation in dermal fibroblast cultures from patients with progressive systemic sclerosis (scleroderma). J. Lab. Clin. Med. **92** : 5–21, 1978
3) Krieg, T., Hörlein, D., Wiestner, M. and Müller, P.K. : Aminoterminal extension peptides from type I procollagen normalized excessive collagen synthesis of scleroderma fibroblasts. Arch. Dermatol. Res. **263** : 171–180, 1978
4) Uitto, J., Bauer, E.A. and Eisen, A.Z. : Scleroderma. Increased biosynthesis of triple-helical type I and type III procollagens associated with unaltered expression of collagenase by skin fibroblasts in culture. J. Clin. Invest. **64** : 921–930, 1979
5) Keiser, H.R. and Sjoerdsma, A. : Direct measurement of the rate of collagen synthesis in skin. Clin. Chim. Acta **23** : 341–346, 1969
6) Barnett, A.J. : Classification and course ; Problems in classification, Scleroderma. (ed. Thomas, C.C.) Springfield, Illinois. 1974, p. 81
7) Juva, K. and Prockop, D.J. : Modified procedure for the assay of H^3-or C^{14}-labeled hydroxyproline. Anal. Biochem. **15** : 77–83, 1966
8) Diegelmann, R.F. and Peterkofsky, B. : Collagen biosynthesis during connective tissue development in chick embryo. Dev. Biol. **28** : 443–453, 1972
9) Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. : Protein measurement with the Folin Phenol reagent. J. Biol. Chem. **193** : 265–275, 1951
10) Perlish, J.S., Bashy, R.I., Stephans, R.E. and Fleischmajer, R. : Connective tissue synthesis by cultured scleroderma fibroblast. I. In vitro collagen synthesis by normal and scleroderma dermal fibroblasts. Arthritis Rheum. **19** : 891–901, 1976
11) Uitto, J., Halme. J., Hannuksela, M., Peltokalio, P. and Kivirikko, K.I. : Protocollagen proline hydroxylase activity in the skin of normal human subjects and of patients with scleroderma. Scand. J. Clin. Lab. Invest. **23** : 241–247, 1969
12) Keiser, H.R., Stein, H.D. and Sjoerdsma, A. : Increased protocollagen proline hydroxylase activity in sclerodermatous skin. Arch. Dermatol. **104** : 57–60, 1971.
13) Winkelmann, R.K. : Pathogenesis and staging of scleroderma. Acta Derm. Venereol. (Stockh) **56** : 83–92, 1976
14) Fleischmajer, R., Perlish, J.S. and Reeves, J.R.T. : Cellular infiltrates in scleroderma skin. Arthritis Rheum. **20** : 975–984, 1977
15) Fleischmajer, R., Damiano, V. and Nediwich, A. : Scleroderma and the subcutaneous tissue. Science **171** : 1019–1021, 1971
16) Johnson, R.L. and Ziff, M. : Lymphokine stimulation of collagen accumulation. J. Clin. Invest. **58** : 240–252, 1976
17) Wahl, S.M., Wahl, L.M. and McCorthy, J.B. : Lymphocyte mediated activation of fibroblast proliferation and collagen production. J. Immunol. **121** : 942–946, 1978
18) Jalkanen, M. and Penttinen, R. : Enhanced fibroblast collagen production by a macrophage-derived factor (CEMF). Biochem. Biophys. Res. Commun. **108** : 447–453, 1982