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Collagen Heterogeneity in Systemic Scleroderma and Other Diseases

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Summary: Proportions of collagen type I and type III were investigated in different tissues (kidney, liver, heart muscle, esophagus, lung and skin) in a single case of malignant systemic scleroderma. The proportion of collagen type III was higher than in healthy controls. Similarly, an increase in the proportion of collagen type III was shown in tissues affected by other inflammatory diseases. Further the authors suggest that the increase in total kidney collagen, especially type III, has important consequences for kidney function; in the described case it had the critical influence on the fatal course of the disease, and the patient died from uremia.

Kollagen-Heterogenität bei systemischer Sklerodermie und anderen Erkrankungen

Zusammenfassung: Die Anteile von Kollagen Typ I und Typ III wurden in verschiedenen Geweben (Niere, Leber, Myokard, Oesophagus, Lunge und Haut) bei einem Fall von maligner systemischer Sklerodermie untersucht. Generell wurde im Vergleich zu Gesunden eine Zunahme des Anteils von Kollagen Typ III festgestellt. Gleichermaßen eine Zunahme des Anteils von Kollagen Typ III findet in Geweben bei anderen entzündlichen Erkrankungen statt. Die Autoren weisen darauf hin, daß die Zunahme des Gesamt-Kollagen, insbesondere des Kollagen Typ III, in der Niere von Bedeutung für die Funktion der Niere ist und daß dies im beschriebenen Fall einen kritischen Einfluß auf den Verlauf der Erkrankung hatte, da der Patient an Uraemie verstarb.

Introduction

Current knowledge of systemic scleroderma extends mainly to the descriptive phase, and our understanding of the disease remains superficial. The skin and subcutaneous elements are not exclusively affected, but the process may be widespread and involve virtually all the tissues of the body. The prognosis of life in the patient with systemic scleroderma depends on the presence and degree of visceral involvement. The most critical organ involvements, which can be fatal in a matter of weeks or several months, are renal or cardiopulmonary. On the other hand esophageal hypomobility is the most common visceral manifestation of scleroderma, and it often appears early. Histologic examinations of systemic scleroderma lesions show alteration of collagen fibers (1). On the other hand the results of different authors (2,3) suggest increased collagen biosynthesis in a number of patients with systemic scleroderma.

Recent advances in collagen chemistry have made possible the detailed characterization of the insoluble fraction. These studies have led to the discovery of collagen heterogeneity. The more abundant type I collagen of molecular composition $(\alpha_1(I))_2\alpha_2$ is present, together with another genetically distinct collagen type III of molecular composition $(\alpha_1(III))_3$ in interstitial tissues. Other genetically distinct collagens are: collagens type IV of composition $(\alpha_1(IV))_3$, and the newly discovered type V from blood vessels, composed of chains αA and αB (4). The occurrence of collagen polymorphism has been identified in different tissues such as skin, aorta, liver, lung, kidney, synovial membrane, peripheral nerve. The content of collagen type II in the loose connective tissue changes in certain physiological and pathological states.

In this report we present data suggesting an increase in the quantity of collagen type III in different tissues

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involved with systemic scleroderma, as well as other sources of inflammatory diseases.

Material and Methods

Samples of tissues

One case of malignant systemic scleroderma was studied. This was a 42 year-old female, in which only six months elapsed between the onset of symptoms and death. In spite of high doses of corticosteroids, the situation of the patient rapidly became worse; urea nitrogen in the blood increased to 119 mg/dl, severe chest pains appeared, extrasystolae were observed in ECG and finally anuria occured before exitus. Ischemic necroses of renal cortex, serofibrinous pericarditis and esophagal erosions, besides others, were found at necropsy.

For comparison skin biopsy was taken from a patient (48 years old) with systemic scleroderma, which was taking a normal course. Cases of bronchopneumonia and a case of liver cirrhosis were also analyzed, in order to study the differences in the respective organs. Further, the composition of collagen was investigated in rheumatoid nodule and in rheumatoid synovial tissue.

Samples of organs under investigation were obtained at necropsy from 3 healthy individuals (46, 53 and 62 years old) that died during traffic accidents. The diagnoses of scleroderma and of rheumatoid arthritis were done according to the A.R.A. criteria (5).

Collagen preparations

Collagens type I and III were isolated by pepsin digestion according to *Fujji & Kühn* (6) and the values were calculated from the hydroxyproline content using correction factors with respect to different hydroxyproline concentration in both collagen types (7.46 for type I and 5.5 for type III).

Separation techniques

Polyacrylamide gel electrophoresis was performed by the modified method of *Stark & Kühn* (7), using β-alanine buffer pH 4.3. The gels were stained with Amido Black 10 B and then evaluated at 680 nm in a Perkin Elmer UV Spectrophotometer Model 402 equipped with a gel scanning accessory.

CM cellulose chromatography (CM cellulose 52 Whatman) was by the original method of *Piez* et al. (8). The column was 1.6 × 6 cm thermostated at 40 °C with a flow rate 72 ml/h. A linear gradient was prepared from two 120 ml quantities of appropriate buffers. The effluent was monitored on a UV spectrophotometer (Perkin Elmer Model 402) using an ultramicro flow-through cell at 230 nm.

Other analyses

Hydroxyproline was assayed according to *Stegeman* (9).

Results and Discussion

Solubility criteria have served for years as the only indicator of differences in collagen from different sources. This included differences between normal tissues, and differences caused by pathological conditions. With the discovery of different collagen types, the question of the relative proportions of these became pertinent.

The main tissues involved in malignant systemic scleroderma were therefore investigated with respect to the content of collagen type III. Normal tissues as well as tissues affected with various diseases served as controls. The identity and purity of isolated collagen type III were proved in all samples by carboxymethyl cellulose chromatography as well as by polyacrylamide gel electrophoresis without and with dithiothreitol pretreatment (fig. 1, 2). Electrophoretic runs revealed the expected increase of the α-band after dithiothreitol treatment. The chromatographic and electrophoretic profiles were in all cases rather similar, and in the figures collagen type III from systemic scleroderma malignant skin is shown as an example.

The data reported here are valid of course for the pepsin solubilized fraction of insoluble collagen, as this solubilization is incomplete in old tissues. According to the results of the methods used the possibility cannot be excluded that small amounts of collagen type I may be present in the fraction precipitated with 1.7 mol/l NaCl; this would be unlikely, however, to have a pronounced effect upon the results. On the other hand basement membrane collagen (type IV) has a precipitation profile similar to that of type III (10), and this might influence the presented data to a greater extent in liver and kidney samples. In healthy tissues the

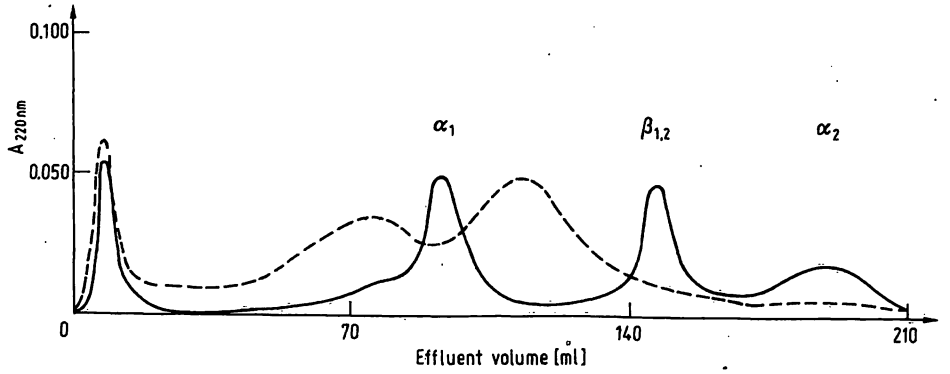

Fig. 1. CM cellulose chromatography of collagen type I (——) and collagen type III (– – – –) Column 1.6 × 6 cm thermostated at 40 °C, flow rate 72 ml/h; linear gradient from two 120 ml quantities of appropriate buffers (8).

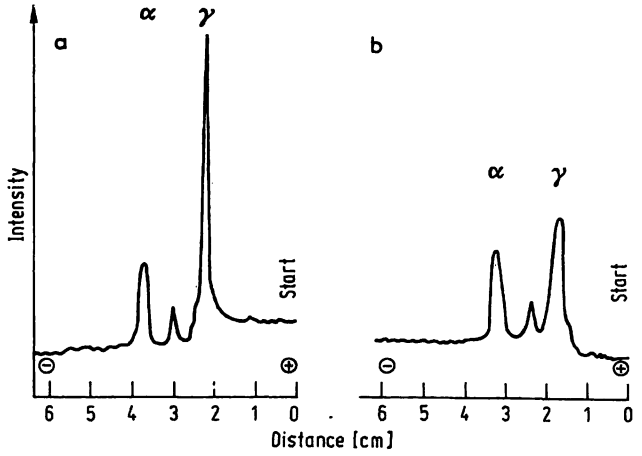

Fig. 2. Densitometric tracing of polyacrylamide gel electrophoresis.
a) without dithiothreitol;
b) after dithiothreitol pretreatment of collagen type III.

lowest amounts of collagen type III occurred in synovial tissue and skin (about 20%), while parenchymatous organs, heart muscle and esophagus constitute about one third of pepsin solubilized collagen (tab. 1). It is to be expected that under normal conditions the amount of collagen type III will vary with age, as shown for skin by *Epstein* (11) and *Deyl & Adam* (12). Furthermore, from the table, it is also evident that collagen type III is increased in inflammed tissues, i.e. in tissues involved with systemic sclerosis as well as with rheumatoid arthritis and bronchopneumonia.

Organ function, especially in parenchymatous organs, is affected not only by the concentration of single collagen types, but also by the total collagen content, which is also increased in diseased tissues. We suggest that this fact is very important for the function of kidney. In the case reported here, collagen exerted an indisputably critical influence on the fatal course of the disease, and the patient died from uremia. As expected from the clinical course and pathological picture, collagen metabolism was also affected in other visceral organs (liver, lung, heart muscle, esophagus).

Of particular interest are collagen changes in liver, where in cirrhosis the amount of collagen type III was lower than in healthy controls. *Fleischmajer* et al. (13) reported that in sclerodermous skin collagen type III is increased mainly in the lower dermis and in subcutaneous tissue.

In general, synthesis of collagen type III predominates in newly formed tissues. *Gay* et al. (14) showed that a single cell is capable of synthesizing both collagen types I and III. In other words it appears that there is a certain category of pathological situations in which the mechanisms determining the type of collagen to be synthesized by a certain population of fibroblasts, or the mechanisms of collagen degradation, are altered. With respect to scleroderma, the protein synthesizing mechanisms must be considered, since it has been shown by *Brady* (15) that in a number of organs in this disease, there is little or no collagenolytic activity.

The actual proportions between individual collagen types, as well as the total amount of collagen, are, of course, influenced by the course of the pathological process. Variations are found in the solubility of skin collagen in scleroderma, and increased as well as decreased collagen concentrations can be found in the soluble fractions (16, 17, 18). In the active stage of scleroderma an increase of collagen biosynthesis has been definitely proved (19), while decreased values have been described in extreme fibrosis.

It is now apparent, that in addition to being the major structural protein, stabilizing body organs by the mechanical strength of its fibers, collagen is also essential for cytodifferentiation, in which it plays a key role. Collagen metabolism and its disturbances are, from this point of view, of crucial importance.

Tab. 1. Collagen type III content from insoluble pepsin-treated collagen in various tissues (in mg/g of total solubilized collagen).

Investigated tissue	Normal 46	53	62 years old	Diseased	
Skin	221	208	191	SSM+	411
				Systemic scleroderma (48 years old)	362
Esophagus	342	332	331	SSM	444
Heart muscle	325	337	301	SSM	408
Liver	391	379	382	SSM	458
				Cirrhosis, late stage (62 years old)	302
Lung	302	288	294	SSM	418
				Bronchopneumonia 1 st case (68 years old)	467
				2nd case (54 years old)	380
Kidney	351	340	312	SSM	430
Synovial tissue	24 years old		204	Rheumatoid arthritis (35 years old)	318
				Rheumatoid nodule (44 years old)	438

+SSM – systemic scleroderma malignant (42 years old)

References

1. Beigelman, P. M., Goldner, F. Jr. & Bayles T. B., (1953), N. Engl. J. Med. *249*, 45–54.
2. Le Roy, E. C., (1972), J. Exp. Med. *135*, 1351–1362.
3. Krieg, T., Müller, P. K. & Goertz, G., (1977), Arch. Dermatol. Forsch. *259*, 105–107.
4. Burgeson, R. E., El Adly, F. A., Kaitila, E. E. & Hollister, D. W., (1976), Proc. Natl. Acad. Sci. U.S.A. *73*, 2579–2583.
5. Bennet, P. H. & Burch, T. A., (1967), Bull. Rheum. Dis. *17*, 453–462.
6. Fujii, T. & Kühn, K., (1975), Hoppe Seyler's Z. Physiol. Chem. *356*, 1793–1801.
7. Stark, M. & Kühn, K., (1968), European J. Biochem. *6*, 534–541.
8. Piez, K. A., Eigner, E. A. & Lewis, M. S., (1963), Biochemistry *2*, 58–66.
9. Stegemann, H., (1958), Hoppe Seyler's Z. Physiol. Chem. *311*, 41–51.
10. Trelstad, R. L., Catanese, V. M. & Rubin, D. F., (1976), Anal. Biochem. *71*, 114–118.
11. Epstein, J. H. Jr., (1974), J. Biol. Chem. *249*, 3225–3231.
12. Deyl, Z. & Adam, M., (1977), Mechanisms Aging Develop. *6*, 25–33.
13. Fleischmajer, R., Gay, S., Meigel, W. N. & Perlish, J. S., (1978), Arthr. Rheum. *21*, 418–428.
14. Gay, H. S., Martin, G. R., Müller, P. K., Timpl, R. & Kühn, K., (1976), Proc. Natl. Acad. Sci. U.S.A. *73*, 4037–4040.
15. Brady, A. H., (1975), J. Clin. Invest. *56*, 1175–1180.
16. Adam, M. & Deyl, Z., (1978), Reumatologia *16*, 75–81.
17. Bashey, R. I., Halpern, S., Stephens, R. E., Perlish, J. S. & Fleischmajer, R., (1975), Biochem. Biophys. Res. Commun. *62*, 303–307.
18. Zimmermann, B. K. & Balda, B. R., (1972), Arch. Dermatol. Forsch. *243*, 357–363.
19. Le Roy, E. C., (1974), J. Clin. Invest. *54*, 880–889.

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