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SHORT COMMUNICATION

Collagen of Dupuytren's disease

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Summary

1. In contrast to collagen from the aponeurosis of normal adult subjects, the nodules, contractures and apparently unaffected aponeurosis from patients with Dupuytren's disease contained substantial amounts of type III collagen.

2. The presence of type III collagen supports the previous proposal that the initial response to injury is the synthesis of an increased proportion of this form of collagen. The increased amounts in the apparently unaffected aponeurosis indicate the disease is not strictly focal but more systemic than is usually considered.

Key words: collagen, cross-links, Dupuytren's disease.

Introduction

Dupuytren's disease involves progressive irreversible contraction of one or more fingers (Dupuytren, 1831; Hueston & Tubiana, 1974; Calnan, 1977). In spite of many studies since the French surgeon Dupuytren established that the affected structure was the aponeurosis, both the aetiology and the pathogenesis of the disease remains obscure.

Macroscopically there is an apparent shrinkage (contracture) of part of the aponeurosis and the formation of nodules in the fascia. The nodules comprise densely packed cells and the contracture consists of dense fibrous connective tissue (Luck, 1959), but no abnormality of the collagen has been reported (Jahnke, 1960; Dahmen, 1961). It has been suggested that myofibroblasts play a role in the contraction of the collagenous tissue (Gabbiani & Majno, 1972).

Histologically there is no real distinction between the macroscopic appearance of scar tissue, keloids and the fibrous tissue in Dupuytren's contracture. Our previous studies on the collagen in human scar tissue (Bailey, Bazin, Sims, LeLous, Nicoletis & Delaunay, 1975a) and granulation tissue indicated a similarity to embryonic collagen. In this paper we report some characteristics of the collagen of Dupuytren's disease.

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Materials and methods

Materials

Specimens were surgically excised from patients with Dupuytren's disease (ages 45–55 years). The samples were further dissected into 'nodules', 'contractures' and apparently 'unaffected' parts of the aponeurosis. Age-matched control samples of aponeurosis were obtained from normal subjects immediately after death.

Pepsin was obtained from Worthington Biochemical Corp. (Freehold, N.J., U.S.A.) (3250 units/mg); KB^3H_4 (650 mCi/mmol) was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). Materials for scintillation fluid were supplied by Nuclear Enterprises (G.B.) Ltd (Edinburgh, Scotland, U.K.).

Acrylamide-gel electrophoresis

The dissected tissues were homogenized, washed in phosphate-buffered NaCl solution (150 mmol/l; pH 7·4) at 4°C for 2 days, and then extracted with acetic acid (0·5 mol/l) at 4°C for 2 days. The insoluble residue was then digested with pepsin, and the solubilized collagen re-precipitated by the addition of NaCl to 0·9 mol/l as described previously (Bailey *et al.*, 1975b).

Samples of the total pepsin digest were examined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, before and after reduction with β-mercaptoethanol, as previously described (Bailey *et al.*, 1975a). Type I collagen is composed of two different peptide chains and has the structure $[\alpha 1(I)]_2\ \alpha 2$, and type III collagen contains three identical peptide chains $[\alpha III]_3$. Owing to the almost similar mobilities of human αIII and α1(I) denatured collagen chains with this technique reduction of the γIII to αIII with β-mercaptoethanol was delayed until 20 min after the start of the electrophoresis, as suggested by Sykes, Puddle, Francis & Smith (1976).

Analysis of stabilizing cross-links

The reducible cross-links were analysed by reduction of the tissue with potassium boro[^{3}H]-hydride and separation of the tritium-labelled compounds by ion-exchange chromatography, as previously described in detail (Robins, Shimokomaki & Bailey, 1973).

Results

Types of collagen

A high proportion of the tissue was solubilized by the pepsin digestion, hence analysis of the soluble collagen can be considered fairly representative of the total collagen. Yields were as follows: 'contractures' 91% (w/w); 'nodules' 81% (w/w); 'unaffected' 75% (w/w); normal 70%.

Electrophoresis of the total pepsin digest revealed as expected that the normal aponeurosis comprised almost pure type I collagen. The granulation tissue-like nodules and contractures showed a significant proportion of type III collagen. Surprisingly the apparently unaffected aponeurosis of the Dupuytren's patient also showed a significant amount of type III collagen.

Confirmation of the identity of type III collagen was obtained by fractional precipitation with NaCl (to 1·5 mol/l), elution position on CM-cellulose and gel-electrophoresis cyanogen bromide pattern.

Reducible cross-links

Distinct differences in the cross-link patterns of the various dissected tissues could be seen. The nodules exhibited a 2·5:1 ratio of dihydroxylysinonorleucine to hydroxylysinonorleucine and a negligible amount of the hexosyl-lysines. The contractures showed a decreased proportion of dihydroxylysinonorleucine, the ratio to hydroxylysinonorleucine being 1·5:1. A significant proportion of the hexosyl-lysines were also present. The apparently unaffected part of the aponeurosis revealed the presence of the reducible cross-links with a similar ratio to the contractures, i.e. 1·7:1, together with a high proportion of hexosyl-lysines. The aponeurosis from a normal subject of the same age gave a pattern typical of mature tissue in that the only major reducible components are the hexosyl-lysines.

The tissues showed a gradual change in pattern, indicating that the nodules were newly formed collagen, the contracture mainly newly synthesized collagen with some mature collagen; the unaffected aponeurosis contained mainly mature collagen, with some newly synthesized, and the normal aponeurosis contained entirely mature collagen.

Discussion

The formation of granulation tissue in the dermis, either during wound healing or in acute and chronic inflammation, results, initially, in synthesis of collagen with properties different from that of the original dermis (Bazin & Delaunay, 1964). Two important features we have previously reported are the greater extent of hydroxylation, resulting in the formation of a more stable cross-link (Bailey, Bazin & Delaunay, 1973), and the increased proportion of type III collagen (Bailey *et al.*, 1975b). Both these variations are typical of embryonic collagen (Bailey & Sims, 1976),

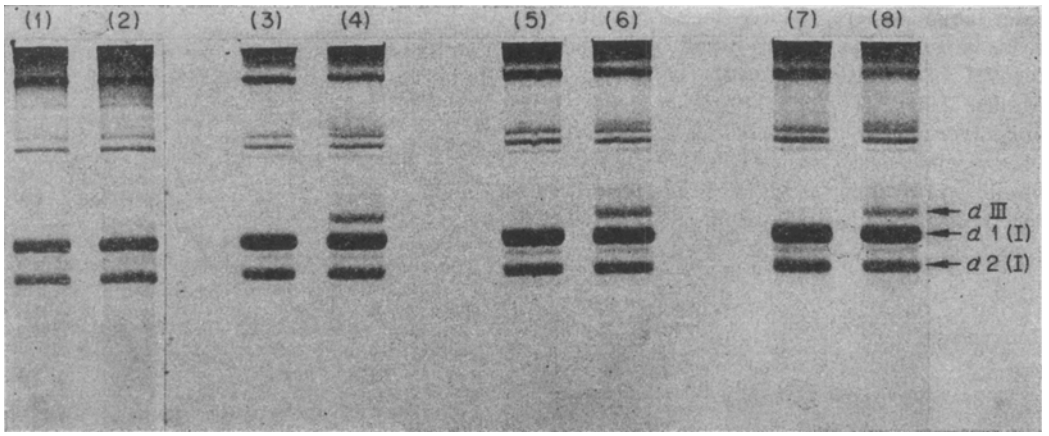

FIG. 1. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the total pepsin digests of tissue* dissected from various sites in the palmar fascia: (1) and (2), aponeurosis from a normal subject without and with reduction by β-mercaptoethanol respectively; (3) and (4), Dupuytren's nodules without and with β-mercaptoethanol respectively; (5) and (6), Dupuytren's contractures without and with β-mercaptoethanol respectively; (7) and (8), 'unaffected' aponeurosis from Dupuytren's patient without and with β-mercaptoethanol respectively. Electrophoresis was interrupted and β-mercaptoethanol added 20 min after the start to resolve αIII and α1(I).

but with increasing time the properties approach those of normal dermal collagen. In contrast to adult skin, which contains an appreciable quantity of type III collagen (10–15%) (Epstein, 1974), the amount of type III collagen in tendon is barely detectable. The increased proportion of type III collagen identified in Dupuytren's disease is therefore more definitive (Fig. 1) and supports our previous proposal that the initial response to injury is the increased synthesis of type III collagen, at least in skin and tendon, although it may be a general phenomenon of injury.

There is little difference in the proportion of type III collagen and the nature of the cross-links between the nodules and the contracture (Fig. 1), both of which are typical of granulation tissues. Surprisingly the apparently unaffected aponeurosis also contained type III collagen, indicating that pathological modification had already started in tissue remote from the contracture. This was supported by the presence of reducible cross-links, an indication of new collagen synthesis. Unlike the nodules and contracture, where, judging from the cross-link patterns, all the collagen appears to be newly synthesized, the unaffected aponeurosis contained significant amounts of the hexosyllysine, which are typical of mature collagen and suggest an early stage of fibrosis. Indeed a few myofibroblasts, which are typical of granulation tissue, could be detected in this area, compared with the many in the Dupuytren's nodules and contracture (Gabbiani & Majno, 1972; Montandon, d'Andiran & Gabbiani, 1977).

It is well known that Dupuytren's disease may affect both palmar aponeuroses and it may also affect the same aponeurosis multifocally (Hueston & Tubiana, 1974). The finding that apparently unaffected areas of aponeurosis from patients with Dupuytren's contracture contain significant amounts of type III collagen as well as myofibroblasts suggests that the disease is more widespread than generally believed.

Recent studies on tendon have shown that small amounts of type III and type IV collagens are present. The type IV collagen from tendon has properties similar to that isolated from human placental basement membrane, and immunofluorescent techniques have revealed that the type III and type IV collagens form a basement membrane sheath, or endotendineum, around the microfibre bundles within the tendon (Duance, Restall, Beard, Bourne & Bailey, 1977). The increased amounts of type III collagen in the 'unaffected' aponeurosis appear by immunofluorescent staining to be confined to these sheaths, which suggests that the initial response to an inflammatory stimulus probably occurs along these endotendineum membranes.

The cells responsible for the increased synthesis of type III collagen could be fibroblasts,

since they have recently been shown in culture to be capable of synthesizing both type I and type III collagens simultaneously (Gay, Martin, Muller, Timpl & Kuhn, 1976). However, a possible role for the myofibroblasts, which are morphologically very similar to cultivated fibroblasts (Gabbiani *et al.*, 1976), and the other cells present in areas of inflammation must be considered.

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