Interactions Between Proteoglycans and Collagen Fibrils in the Palmar Fascia in Dupuytren's Disease

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Introduction

In Dupuytren's disease the morphology and function of palmar fascia change resulting in a progressive and irreversible flexion of the fingers. The pathogenesis is not known, although many efforts has been made to describe the histology and biochemistry of Dupuytren's tissue. In the nodules, the cells proliferate and differentiate to myofibroblasts (Gabbiani and Majno 1972, reviewed by Schürch et al. 1992) and the amount and types of collagen and glycosaminoglycans are altered (Bailey et al. 1977; Bazin et al. 1980; Brickley-Parsons et al. 1981; McFarlane et al. 1990; Gelbermann et al. 1980; Hanyu et al. 1984; Hunter et al. 1975; Meister et al. 1979; Menzel et al. 1979; Tunn et al. 1988). Later, tendon-like cords are formed; these contain only a few fibroblasts in an excessive collagenous matrix (Luck 1959; Chiu and McFarlane 1978).
In the present investigation the extracellular matrix of the normal and diseased palmar fascia were compared. The proteoglycans were stained by two different histochemical procedures: (1) acridine orange, according to the method of Shepard and Mitchell (1981) and (2) reduced osmium tetroxide, according to the method of Karnovsky (1971). The localization of the reaction products in the matrix and their interaction with the collagen fibrils were analyzed and compared. The alterations seen during the disease demonstrate the importance of these proteoglycans in the correct function of the palmar fascia. Preliminary results have been published (Brandes et al. 1991a).

Materials and Methods

Specimens from four individuals with normal palmar fascia and from 20 men (mean age 50 years) with Dupuytren's contracture were fixed in 3% glutaraldehyde in 0.1 *M* sodium cacodylate buffer (pH 7.2) immediately after surgery. After rinsing in the same buffer the specimens were placed in 1% osmium tetroxide and 1.5% potassium ferrocyanide in water (Karnovsky 1971). Other specimens were fixed in aldehyde with addition of 0.01% acridine orange (Polysciences, Warrington, USA), their postfixation was performed in 2% osmium tetroxide in 0.1 *M* cacodylate buffer. All the specimens were

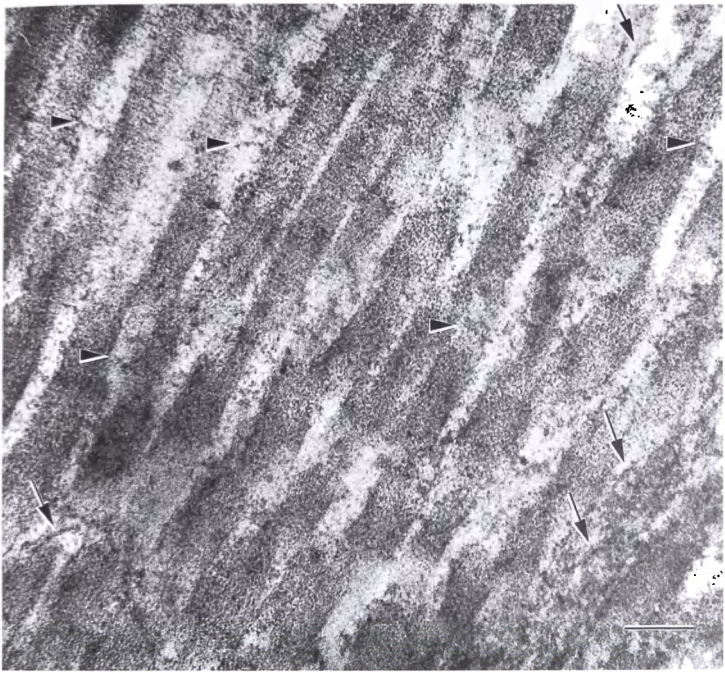
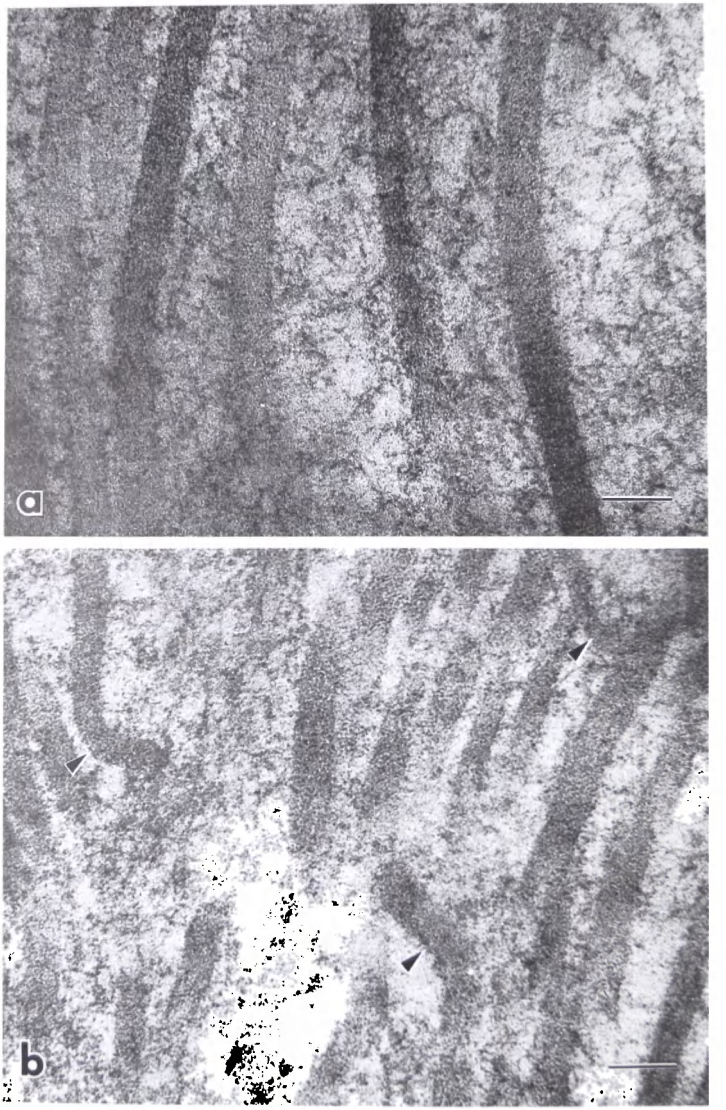


Fig. 1. Normal palmar fascia fixed in the presence of acridine orange. Parallel oriented collagen fibrils are surrounded by fine reaction products appearing as short (*arrowheads*) and long (*arrows*) filaments. The long filaments form a large interfibrillar assembly on the *right* side of the micrograph. *Bar* = 0.1 μm; ×120 000

dehydrated in graded alcohols and embedded in Epon. Thin sections, stained with uranyl acetate and lead citrate, were examined in a Siemens Elmiskop 101 electron microscope.

Results

In the normal palmar fascia, the fibroblasts were embedded in an extracellular matrix containing parallel bundles of collagen fibrils with a diameter of about 50–80 nm connected to each other by 3–4 nm thick, short, filamentous reaction products. These were orthogonally oriented along the fibrils at distances of 10–25 nm (Fig. 1, 3b). Long branched filaments with a similar thickness and a length of about 100 nm lay mainly parallel to the collagen fibrils and formed a network in the broader interfibrillar spaces (Fig. 1).


a
b

In the Dupuytren's disease fascia, the nodules were characterized by a predominance of myofibroblasts. These cells were surrounded by a tight network of 2–3 nm thick and about 100 nm long branched filaments. They bound at distances of 10–25 nm to collagen fibrils, about 40–60 nm thick, which were intermingled with the network (Fig. 2a). At greater distances from the cells, the collagen fibrils were very irregularly oriented, abruptly changing their course but packed closer together than in the pericellular matrix (Fig. 2b). They were surrounded by abundant, short fine filaments connected to the fibrils at distances of 10–25 nm (Fig. 2b).

The cords contained only a few fibroblasts, which were closely packed between large bundles of collagen fibrils. After fixation with glutaraldehyde and acridine orange, the fibrils disaggregated into helicoidal microfibrils (Fig. 3a). Therefore, the diameter of the fibrils measured from 350 nm to 850 nm. The interfibrillar spaces disappeared. A fine network stained by acridine orange could only be seen within the fibrils (Fig. 3a). After using reduced osmium tetroxide the swelling of the collagen fibrils was limited. Fine filaments extended from the narrow interfibrillar spaces into the outer zone of each collagen fibril (Fig. 4).

Discussion

The present study demonstrates that the extracellular matrix changes both qualitatively and quantitatively during the course of Dupuytren's disease. Whereas in the ultrastructural analysis the collagen fibrils could be seen with the usual preparation techniques, special methods were needed to visualize the proteoglycans. Acridine orange and reduced osmium tetroxide can be used to localize sulfated glycosaminoglycans as demonstrated in hyaline cartilage by digestion with specific enzymes (Brandes and Reale 1988, 1990). Therefore, the fine filamentous reaction products in the palmar fascia are presumably proteoglycans.

In the nodule the matrix around the myofibroblasts usually contains predominantly proteoglycans which connect the cells with the collagen fibrils and these to each other (Badalamente et al. 1983; Chiu and McFarlane 1978; Gabbiani and Majno 1972). Therefore, contraction of the myofibroblasts seems to cause the parallel orientation of the newly formed collagen fibrils (Badalamente et al. 1983; Brickley-Parsons et al. 1981; Chiu and McFarlane 1978). Additionally, the proteoglycans themselves have an important influence on the cells and matrix (reviewed by Ruggeri and Benazzo 1984): First, due to their high water-binding capacity, they fill the whole matrix regulating the

Fig. 2a,b. Dupuytren's disease. In the nodule close to the myofibroblasts (**a**) a tight filamentous network can be seen in which the collagen fibrils are intermingled. Far away from the cells (**b**) prevailing short filaments connect the fibrils which are packed closer together and are more disorderly than in the pericellular matrix. *Arrowheads* indicate fibrils following an irregular pathway. *Bar* = 0.1 μm; ×120000

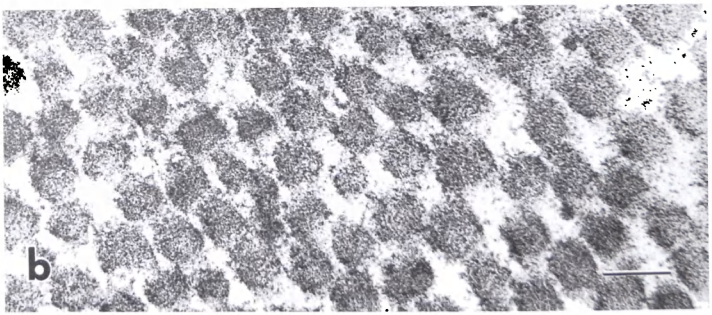


Fig. 3. a Dupuytren's disease, cord. After fixation in the presence of acridine orange the collagen fibrils are swollen. A finely stained network (*arrowheads*) lies between the helicoidal microfibrils. **b** Normal palmar fascia fixed in the same solution as in **a**. *Bar* = 0.1 μm; ×120 000

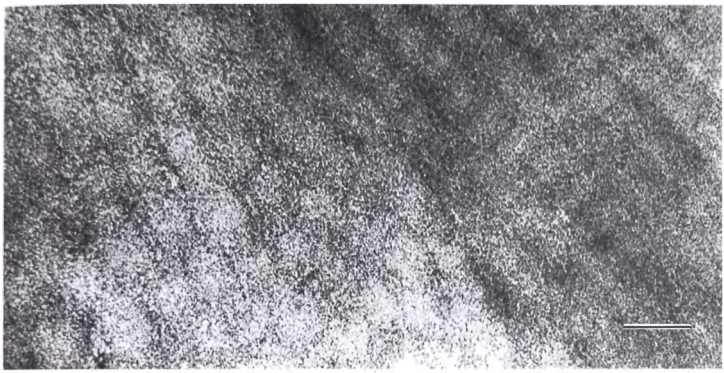


Fig. 4. Dupuytren's disease, cord. Reduced osmium tetroxide induces a limited swelling of the collagen fibrils. Staining is present between and within the outer zone of the collagen fibrils. *Bar* = 0.1 μm; ×120000

transport of substances to the cells and of cell products to the surrounding matrix. Second, they influence fibrillogenesis, as shown by in vivo and in vitro experiments.

During the pathological process not only the amount but also the type of the proteoglycans changes. In the normal palmar fascia hyaluronan and dermatan sulfate predominate; in Dupuytren's tissue hyaluronan decreases, and chondroitin sulfate increases more than dermatan sulfate in the nodule but does not change quantitatively in the cords (Flint et al. 1982; Gurr et al. 1984; Tunn et al. 1988). Based on comparing the reaction products seen after staining with acridine orange or reduced osmium tetroxide, the shorter filaments connected to the collagen fibrils could be composed of dermatan sulfate proteoglycans, since they are present in the normal and diseased fascia. The tight network of long branched filaments predominantly seen in the nodule around the myofibroblasts could contain most of the chondroitin sulfate, which reacts with acridine orange and with reduced osmium tetroxide. Although this hypothesis has to be proven by digestion with specific glycosaminoglycan lyases, it is in agreement with the results on other connective tissues (reviewed by Ruggeri and Benazzo 1984).

The functional characteristics of the connective tissue are mainly affected by proteoglycans and collagen: The small dermatan sulfate proteoglycans seem to have an important role in the fibrillogenesis and alignment of the newly formed collagen fibrils, for example, in the tendon (Öbrink 1973; Ruggeri and Benazzo 1984; Scott 1980) and probably in the normal palmar fascia as well. In comparison, the large chondroitin sulfate proteoglycans of the hyaline cartilage and also of Dupuytren's nodule fill the broad interfibrillar spaces and are typically found with small, randomly oriented collagen fibrills. These macromolecules are responsible for the resilience of the tissue: acting pressure

forces the water between the glycosaminoglycan chains to be squeezed out and the fibrillar constituents to be stretched. Since after unloading the proteoglycans can again bind water, the tissue recovers to its previous state. Therefore, the functional characteristics of the nodule of Dupuytren's disease can be explained by its cartilage-like appearance, which is caused by a specific and mutual interaction between proteoglycans and collagen fibrils.

In the cords, where only a few proteoglycans can be recognized in the interfibrillar spaces, the collagen fibrils are swollen, probably through the prevailing intrafibrillar proteoglycans (Brandes et al. 1991a). Conceivably, the balance between interfibrillar and intrafibrillar proteoglycans is important for the buildup and maintenance of the collagen fibrils in the extracellular matrix of the palmar fascia.

In summary, the present study demonstrates that interaction of the different proteoglycans with collagen fibrils can explain the morphological and functional changes of the palmar fascia during Dupuytren's disease. Conclusions regarding the pathogenesis remain difficult, since this tissue retains its ability to adapting to acting forces (Brandes et al. 1991b).

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