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J Hand Surg [Br] 1996 21: 801
DOI: 10.1016/S0266-7681(96)80196-3

CONTRACTION OF COLLAGEN LATTICES BY CELLS FROM DUPUYTREN'S NODULES

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The aim of this study was to see if nodular cells in Dupuytren's disease differed from dermal cells in their contractile capacity and motility. Ten surgical specimens from patients with Dupuytren's disease and contracture of the finger of more than 45° were harvested and the nodular cells were explanted and cultured. Dermal fibroblasts from the forearm were used as control cells. Both types of cell had the same growth pattern. The morphology on confocal laser scanning microscopy was also similar in both types of cell. Dermal control cells caused significantly more contraction of collagen lattices compared with fibroblasts from nodules of Dupuytren's contracture. The F-actin content was equal in both groups. Platelet derived growth factor, PDGF-BB (but not PDGF-AA), increased the chemotactic activity of both cell types, but there were no differences between them. The results indicate that at a late state of the disease cells from Dupuytren's nodules lose their contractile capacity and regain a phenotype resembling that of dermal fibroblasts.

The Journal of Hand Surgery (British and European Volume, 1996) 21B: 6: 801–805

Pathological contraction of connective tissue is the central phenomenon in Dupuytren's disease. In the early stage of the disease nodules are formed by aggregation of proliferated fibroblasts that adhere to fibrin (Merlo et al, 1986). The fibroblasts undergo a phenotypic change and the intracellular filamentous actin (F-actin) increases with the formation of myofibroblasts (Foo et al, 1992; Jester et al, 1986; Oda et al, 1988; Pasquali-Ronchetti et al, 1993; Vande Berg et al, 1984). These myofibroblasts are concentrated in the nodules whereas the fibrotic cords have only a few fibroblast-like cells (Pasquali-Ronchetti et al, 1993). The presence of myofibroblasts in nodules of Dupuytren's contracture, together with several ultrastructural and immunochemical similarities with smooth muscle cells, supports the idea that myofibroblasts have a key role in fibrocontractive diseases (Foo et al, 1992; Gabbiani, 1992; James and Odom, 1980; Jester et al, 1986; Pasquali-Ronchetti et al, 1993). A more recent theory about contraction is that fibroblasts move around in the extracellular matrix and rearrange the collagen fibres in a manner that results in contraction of the surrounding tissue (Andujar et al, 1992; Ehrlich and Rajaratnam, 1990; Harris et al, 1981).

Platelet derived growth factor (PDGF) is a dimeric growth factor composed of A and B chains (Soma et al, 1992). These chains act through specific receptors on the cell's surface, which have differential ligand binding properties for the PDGF isoforms (AA, AB, BB) and which regulate the biological responsiveness of cells to PDGF. The isoform AA is the main PDGF dimer that is contained in human platelets and at sites of injury during the acute phase of wound healing (Soma et al, 1992).

The aim of our study was to investigate the contractile capacity and motility of fibroblasts from nodules of patients with late stage Dupuytren's contracture in response to PDGF. The F-actin content of the fibroblasts and their morphology were assessed with flow cytometry and confocal laser scanning microscopy, respectively.

MATERIALS AND METHODS

Cells from 10 patients operated on for Dupuytren's contracture were explanted and cultured. The indication for operation was a digital contracture of more than 45°. All patients had areas of palpable thickening within the fascia which were regarded as nodules. Dermal control cells from the forearm of all patients were harvested and cultured. The study was approved by the ethics committee for human experimentation at Linköping University.

Tissue culture methods

Specimen from nodules of Dupuytren's contracture were cut into pieces 1×1 mm in area and placed in dishes 100 sq cm in area. After 5 to 10 minutes the explants had attached to the bottom of the dish. We then added 15 ml of Eagle's minimum essential medium (EMEM) and 20% heat-inactivated newborn calf serum, 2 ml of L-glutamine, and 2 ml of a solution containing penicillin (100 U/ml) and streptomycin (100 µg/ml) to the dishes. Cultures were maintained in a humidified atmosphere containing 5% CO_2 at 37°C. After 10 days the cultures were trypsinized and transferred to flasks 25 sq cm in area with 7 ml of culture medium. At confluence the cultures were trypsinized and cells allocated to two flasks (passage 1). All experiments were done during the fourth to sixth passages.

Preparation of collagen lattices

Collagen type I was extracted from rat tendons as described by Bell et al (1979), with minor modifications. Ligaments from six rat tails were placed in 300 ml

diluted acetic acid (1/1000). The mixture was stirred for 48 hours at 4°C and centrifuged at 26100 r.p.m. for 1 hour. The collagen concentration in the aqueous stock solution was adjusted to 2.5 mg/ml. We mixed 1 ml of chilled serum-free medium with 0.5 ml of chilled collagen solution in a 35 mm Petri dish and added 0.5 ml of chilled freshly-trypsinized fibroblasts diluted with serum free medium to 2×10^5 cells/lattice. Six lattices were prepared for each patient. Samples of serum and medium were bought from Labdesign, Täby, Sweden. The dishes were kept in an incubator with 5% CO_2 and 95% air at 37°C. After 10 minutes the gels were detached by gently shaking the dishes. After 36 hours images of the lattices were made using a Hamamatsu CCD video camera connected to a Macintosh computer. The area of the lattices were estimated using the image analysing program NIH Image 1.54.

Actin staining and flow cytometry

Freshly released cells from tissue flasks were dispersed in serum free medium (10^5 cells/ml), centrifuged and fixed in 0.5% paraformaldehyde for 30 minutes. A total of 1 ml of citrate buffer containing 0.6 μg phalloidin FL (Bodipy®, Molecular Probes, Eugene, Oregon, USA) was added to the pellet for 10 to 20 minutes. The solution was filtered through a nylon mesh with a pore size of 41 μm after which 15 μg propidium iodide (Labkemi, Stockholm, Sweden) was added. The actin content was measured with an FACS flow cytometer (Becton Dickinson) equipped with an argon laser (488 nm) to activate phalloidin and propidium iodide simultaneously. Ten thousand events were recorded.

Confocal laser scanning microscopy

After we had estimated the area of the lattices, the cells were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) for 10 to 20 minutes and stored in PBS. The F-actin was stained with phalloidin 0,6 μg/lattice for 20 minutes. The morphology of the fibroblasts was studied with a Zeiss LSM 410 inverted confocal laser scanning microscope at an emission fluorescence of 510 to 525 nm after activation with a 488 nm laser beam.

Chemotaxis assay

The chemotactic response of fibroblasts from four patients with Dupuytren's contracture to both PDGF-AA and -BB was assayed with a two chamber system, Blind-Well®, (Labdesign, Täby, Sweden). All experiments were done in quadruplicate. PDGF-AA (8.4 ng/ml) and PDGF-BB (20 ng/ml; Sigma, St Louis, USA) were diluted in EMEM containing bovine serum albumin (BSA) 0.2 mg/ml and put in the lower well of the Blind-Well® chamber. The cells (2×10^5 cells/ml) were dispersed in EMEM that contained BSA 2 mg/ml and fibronectin 5 μg/ml (Labkemi, Stockholm, Sweden) and put in the upper well. The solutions in the wells were in contact with each other through a collagen coated polycarbonate filter, Nuclepore® (Labdesign, Täby, Sweden), with 8 μm pores. The chambers were kept at 37°C for 4 hours in a humidified incubator that contained 5% CO_2. The filters were removed and the attached cells fixed and stained with Diff Quik stain (Labex, Helsingborg, Sweden). The cells from the upper surface of the filters were removed by scraping with a rubber spatula. The migrated cells on the lower surface of the filter were quantitated by extracting the stain with 210 μl 0.1 M hydrochloric acid for 15 minutes. A total of 150 μl of that solution was placed in a 96-well ELISA plate with a flat bottom. The absorbency at 620 nm was measured spectrophotometrically.

Statistical analysis

All statistics were calculated using the STATISTICA software package (StatSoft, Inc.). We compared the difference in contraction between the two types of cell by analysis of variance. The significance of differences in the actin content was assessed by the two-tailed Student's *t*-test. As the one-way ANOVA gave $F = 0.71$, the differences between patients were disregarded and the significance between the chemotactic activity to PDGF was assessed with Student's *t*-test.

RESULTS

The cells from Dupuytren's nodules and the control dermal cells had the same pattern of growth and both reached confluence after 12 days. On confocal laser scanning microscopy both types of cell had the same appearance (Fig 1). Both types of tissue contained few spindle-shaped cells with long thin extensions which here and there seemed to be in contact with each other. By rotating a scanned three-dimensional picture we confirmed the impression of intercellular contacts that formed a cellular network.

Control dermal cells caused 35% contraction of the lattice area compared to 19% by cells from Dupuytren's contractures ($P < 0.01$; Fig 2). The F-actin content estimated by flow cytometry was similar in both types of cells (Fig 3). PDGF-AA had no effect on the chemotactic activity of cells from Dupuytren's disease or dermis (Fig 4a). PDGF-BB added to the lower chamber increased chemotaxis equally in both types of cells ($P < 0.001$; Fig 4b).

DISCUSSION

Our main finding was the significantly increased contractile capacity of regular dermal fibroblasts of the forearm compared with cells from Dupuytren's nodules. The natural history of Dupuytren's contracture can be divided into proliferative, involutional and residual phases.

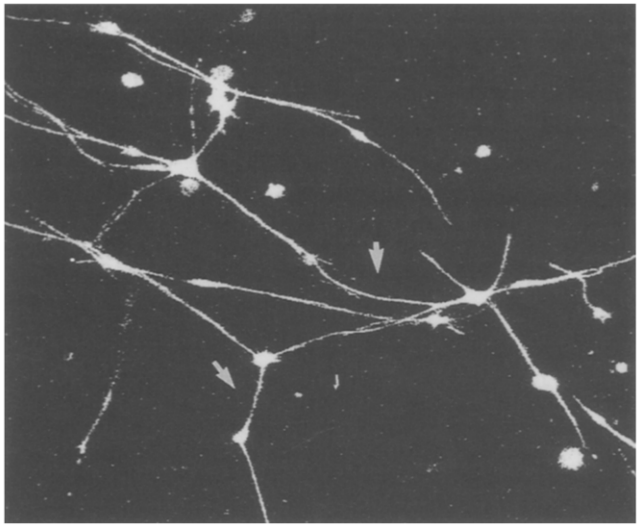

Fig 1 Three-dimensional image with confocal laser scanning microscopy of cells from Dupuytren's nodules. Note the thin extensions which are in contact with each other (arrow). Original magnification × 40.

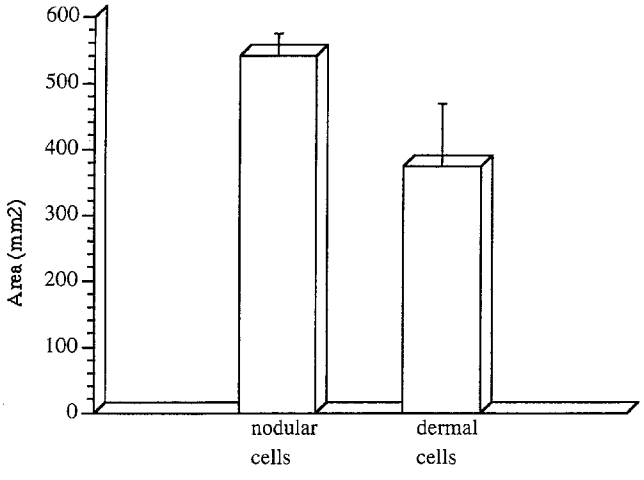

Fig 2 The contraction of collagen lattices by dermal (control) and cells from Dupuytren's nodules ($P < 0.01$). Mean (SEM).

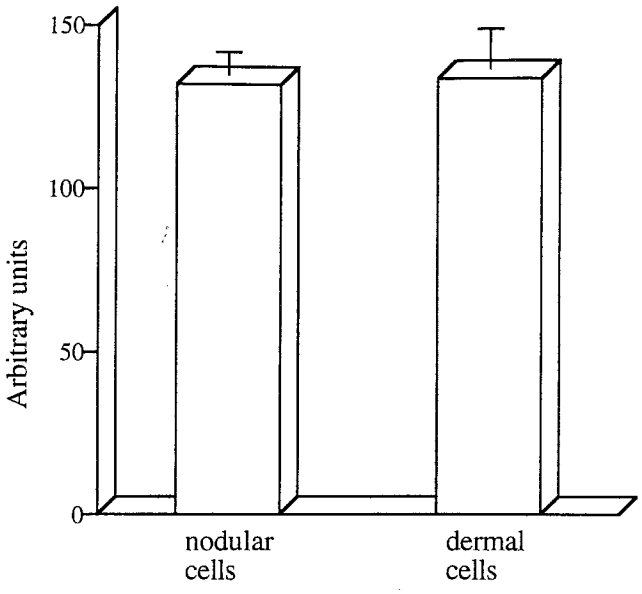

Fig 3 The actin content of palmar fascia of patients with Dupuytren's contracture and of dermal cells (control) given as arbitrary units. Mean (SEM).

Nodules are formed during the proliferative phase and it is believed that the contraction of the palmar fascia starts during this phase. The predominant type of cells found in the involutional phase are myofibroblasts, with microfilamentous bundles that have the immunohistochemical and ultrastructural properties of actin and a slower growth pattern than palmar dermal cells (Vande Berg et al, 1984). This cell type seems to play a key role in the contraction (Schürch et al, 1990; Gabbiani, 1992). The nodules which are rich in myofibroblasts are thought to be the active source of contracture (Vande Berg et al, 1982). As the indication for operation in this study was digital contraction of more than 45° (late stage) the finding of similar growth patterns and actin content in nodular and dermal cells indicates that nodular myofibroblasts may be transitional cells, the number of which decreases as the disease progresses. This is in agreement with other reports of the absence of myofibroblasts during the residual phase of the disease (Gokel and Hübner, 1977; Merlo et al, 1986; Rudolph and Vande Berg, 1991). However, it has been reported that cells from Dupuytren's nodules of patients who had been operated on for digital contraction of more than 30° are composed almost entirely of myofibroblasts (Vande Berg et al, 1984; Foo et al, 1992). Although myofibroblasts are regarded as key cells in the contractile phenomenon (Gabbiani, 1992), they have not always been found in contracted palmar fascia (Vande Berg et al, 1982; Badalamente et al, 1983; Tomasek et al, 1986; Pasquali-Ronchetti et al, 1993).

The exact mechanism of contraction is still unknown. One theory is based on the appearance of myofibroblasts in contracting tissue (Badalamente et al, 1983; Vande Berg et al, 1984; Gabbiani, 1992). Ultrastructurally, these cells resemble smooth muscle cells and it is proposed that the intracellular stress fibres that are rich in fibrillar-actin contract, which in turn makes the cells contract the surrounding connective tissue through their intercellular contacts (Rudolph and Vande Berg, 1991). Biochemically, however, there are dissimilarities between myofibroblasts and smooth muscle cells. Tomasek and Haaksma (1991) showed by indirect immunofluorescence that nodular Dupuytren's contracture cells lacked smooth-muscle-type myosin and the smooth-muscle-associated extracellular laminin. On confocal laser scanning microscopy we found that the cells formed a syncytium by intercellular connections and this confirmed earlier ultrastructural observations (Pasquali-

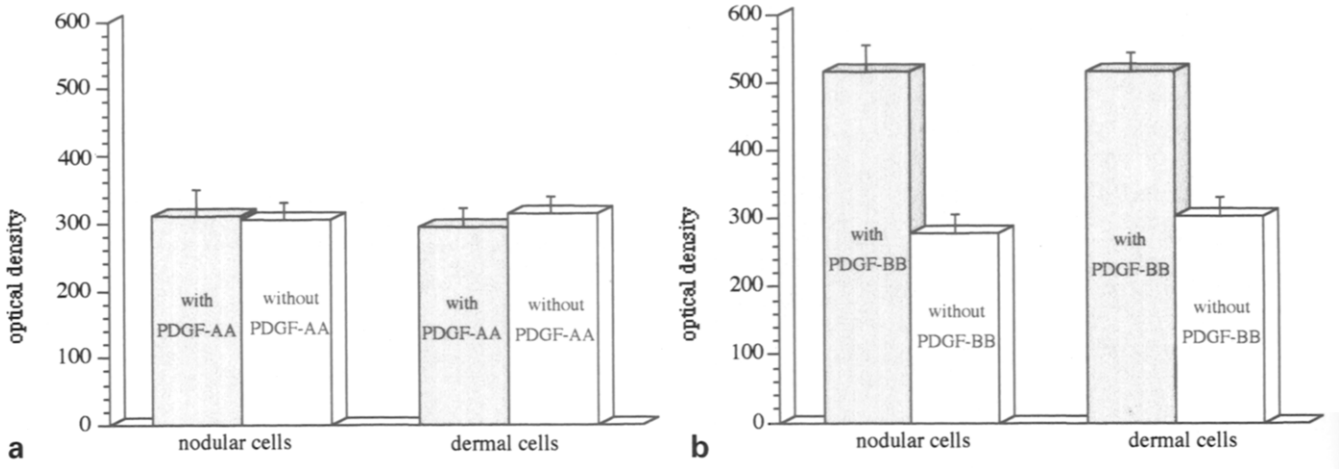

Fig 4 The chemotactic activity of (a) PDGF-AA and (b) PDGF-BB on dermal and Dupuytren's contracture cells. PDGF-BB (but not PDGF-AA) caused a significant increase in chemotactic activity of both cell types ($P < 0.001$). Mean (SEM).

Ronchetti et al, 1993). Our finding of similar amounts of actin in Dupuytren's contracture and dermal fibroblasts is not in accordance with the theory of multicellular contraction, according to which one would have expected a higher content of actin in dermal cells. However, we found no increase in the F-actin content of dermal cells.

Another theory of contraction is that cell locomotion causes collagen fibres to reorganize in a manner that contracts surrounding connective tissue (Ehrlich, 1988; Andujar et al, 1992). We therefore studied the effect of PDGF on the motility of fibroblasts to see if the increased contractility of dermal cells could be the result of an increased reactivity to PDGF implemented by an increased number of membrane receptors to the growth factor. Despite the fact that PDGF-AA is the predominant isoform in human platelets (Soma et al, 1992) it had no chemotactic effect on either cell type. A difference in cell motility induced by the two PDGF isoforms has recently been reported, as stimulation of serum-starved human fibroblasts (AG 1523) with PDGF-BB (but not PDGF-AA) induced circular ruffles and the outgrowth of lamellipodia on the cell surface (Lassing et al, 1994).

One explanation for the minor degree of contraction of cells from Dupuytren's contractures could also be that during the cell culturing process they undergo more changes in their phenotype than the dermal cells. It has been shown, however, that cultured cells from Dupuytren's nodules maintain their differentiated state as myofibroblasts through five passages (Vande Berg et al, 1984) and not until passage 15 do cells degenerate and cease to grow (Rudolph and Vande Berg, 1991). All our experiments were done at passages 4 to 6 and we therefore do not think that the culturing procedures resulted in changes of the contractile capacity of Dupuytren's contracture-cells.

The results of this study suggest that fibroblasts from the final phase of Dupuytren's contracture have a similar actin content and the same chemotactic activity to PDGF as normal dermal fibroblasts. In contrast, the dermal fibroblasts contract collagen gels significantly better than Dupuytren's contracture fibroblasts. It is possible that the contractile capacity of the aponeurotic Dupuytren's contracture-cells is reduced as the disease progresses. One interesting question is whether the nodular cells permanently lose their contractile capacity or if they can regain their differentiated state as contractile cells.

Acknowledgements

The study was supported by grants from the Swedish Medical Research Council (Project No. 9881), the Swedish Society of Medicine (Project No 953.0), and the County Council of Östergötland, Sweden.

References

ANDUJAR M B, MELIN M, GUERRET S and GRIMAUD J A (1992). Cell migration influences collagen gel contraction. Journal of Submicroscopic Cytology and Pathology, 24: 145–154.

BADALAMENTE M A, STERN L and HURST L C (1983). The pathogenesis of Dupuytren's contracture: contractile mechanisms of the myofibroblasts. Journal of Hand Surgery, 8: 235–243.

BELL E, IVARSSON B and MERRILL C (1979). Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. Proceedings of National Academy of Science. USA, 76: 1274–1278.

EHRLICH H P (1988). Wound closure: evidence of cooperation between fibroblasts and collagen matrix. Eye, 2: 149–157.

EHRLICH H P and RAJARATNAM J B M (1990). Cell locomotion forces versus cell contraction forces for collagen lattice contraction: an in vitro model of wound contraction. Tissue and Cell, 22: 407–417.

FOO I H, NAYLOR I L, TIMMONS M J and TREJDOSIEWICZ L K (1992). Intracellular actin as a marker for myofibroblasts in vitro. Laboratory Investigation, 67: 727–733.

GABBIANI G (1992). The biology of the myofibroblast. Kidney International, 41: 530–532.

GOKEL J M and HÜBNER G (1977). Occurrence of myofibroblasts in different phases of morbus Dupuytren (Dupuytren's Contracture). Beitrage zur Pathologie, 161: 166–175.

HARRIS A K, STOPAK D and WILD P (1981). Fibroblast traction as a mechanism for collagen morphogenesis. Nature, 290: 249–251.

JAMES W D and ODOM R B (1980). The role of the myofibroblast in Dupuytren's contracture. Archives of Dermatology, 116: 807–811.

JESTER J V, RODRIQUES M M and HERMAN I M (1986). Characterization of avascular corneal wound healing fibroblasts. American Journal of Pathology, 127: 140–148.

LASSING I, MELLSTRÖM K and NISTÉR M (1994). Comparison of PDGF-AA and PDGF-BB-induced phosphoinisitide formation in human and mouse fibroblasts. Experimental Cell Research, 211: 286–295.

MERLO G, AMBROGGIO G P and CASTAGNA B (1986). Fibrin/fibrinogen and fibrolytic activity of the palmar fascia. Journal of Hand Surgery, 11B: 55–59.

ODA D, ALLEN M, GOWN A M, VANDE BERG J S and STERN R (1988). The fibroblast-like nature of myofibroblasts. Experimental and Molecular Pathology, 49: 316–329.

PASQUALI-RONCHETTI I, GUERRA D, BACCARANI-CONTRI M et al (1993). A clinical, ultrastructural and immunochemical study of Dupuytren's disease. Journal of Hand Surgery, 18B: 262–269.

RUDOLPH R and VANDE BERG J (1991). The myofibroblast in Dupuytren's contracture. Hand Clinics, 7: 683–693.

SCHÜRCH W, SKALLI O and GABBIANI G. Biology and treatment. In: *Dupuytren's disease*. McFarlane R M, McGrouther D A and Flint M H (Eds): Edinburgh, Churchill Livingstone, 1990: 31–47.

SOMA Y, DVONCH V and GROTENDORST G R (1992). Platelet-derived growth factor AA homodimer is the predominant isoform in human platelets and acute human wound fluid. FASEB Journal, 6: 2996–3001.

TOMASEK J, SCHULTZ R S, EPISALLA C W and NEWMAN S A (1986). The cytoskeleton and extracellular matrix of the Dupuytren's disease myofibroblasts: an immunofluorescence of a nonmuscle cell type. Journal of Hand Surgery, 11A: 365–371.

TOMASEK J J and HAAKSMA C J (1991). Fibronectin filaments and actin microfilaments are organized into a fibronexus in Dupuytren's diseased tissue. Anatomical Record, 230: 175–182.

VANDE BERG J S, RUDOLPH R, GELBERMAN R and WOODWARD M R (1982). Ultrastructural relationship of skin to nodule and cord in Dupuytren's contracture. Plastic and Reconstructive Surgery, 69: 835–844.

VANDE BERG J S, GELBERMAN R H, RUDOLPH R, JOHNSON D and SICURELLO P (1984). Dupuytren's disease: comparative growth dynamics and morphology between cultured myofibroblasts (nodule) and fibroblasts (cord). Journal of Orthopaedic Research, 2: 247–256.

Received: 14 December 1995
Accepted after revision: 3 June 1996
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