

# Prostaglandins influence myofibroblast contractility in Dupuytren's disease

This study investigated if the vasoactive prostaglandins, PGE<sub>2</sub>, and PGF<sub>2α</sub>, were identifiable in association with nodular myofibroblasts of patients with Dupuytren's disease. Immunocytochemic studies, using antibodies specific for these prostaglandins, have confirmed their association with myofibroblasts. Radioimmunoassay was used to quantitate the prostaglandins. Our results indicate a significant increase of both prostaglandins, especially PGF<sub>2α</sub>, in Dupuytren's palmar fascia when compared with control fascia. These endogenous prostaglandins may influence the contractile behavior of myofibroblasts in Dupuytren's disease to contribute to the pathobiology of this disorder. (J HAND SURG 1988;13A:867-71.)

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The morphologic and collagen biochemical abnormalities associated with the progressive flexion deformity of one or more digits—known as Dupuytren's disease—has been extensively studied. The work of Gabbiani and Majno<sup>1</sup> has defined the myofibroblast as the dominant cell type associated with the pathognomonic nodule of the disease. The reports of a series of investigators<sup>2-5</sup> have documented increased amounts of type III collagen and hexosamine glycosaminoglycan in affected palmar fascia. However, to date, the connection between the development of the clinical, progressive flexion contractures of the digits and these biochemical abnormalities has not been explained.

It has been generally shown that the myofibroblast is a contractile cell.<sup>1, 6, 7</sup> In our laboratory we have identified part of the intracellular contractile mechanism of myofibroblasts by demonstrating the enzyme, calcium adenosinetriphosphatase (ATPase), in association

with intracellular filaments of these cells.<sup>6</sup> This enzyme is essential for the contraction of muscle cell types.

However, there has been debate concerning the possible transmission of cellular contractile forces by myofibroblasts to surrounding collagen as an etiologic hypothesis in producing flexion deformities in Dupuytren's disease. We have previously demonstrated glycosaminoglycan fibrils that formed connections between myofibroblasts, and from myofibroblasts to collagen.<sup>6</sup> Recent studies in wound closure,<sup>8</sup> as well as in Dupuytren's disease,<sup>9</sup> have shown that fibronectin strands, 2 to 5 nm in diameter, and fibrils within the extracellular matrix interconnect myofibroblasts and connect the termini of the filament bundles within myofibroblasts to collagen fascicles in the extracellular space.<sup>9</sup> These authors suggest that these cell to cell connections and cell to collagen connections may account for the transmission of myofibroblast contractile forces required for collagen's physical deformation, subsequently resulting in the flexion deformity of the disease.

Therefore, investigation of factor(s) that might modulate myofibroblast contractility behavior appear to be important. Myofibroblasts resemble smooth muscle cells, especially vascular endothelial cells. Therefore, we decided to test the in vitro response of Dupuytren's myofibroblasts to prostaglandins (PG) capable of inducing vasoconstriction and vasodilation (PGF<sub>2α</sub> and PGE<sub>2</sub>, respectively). The results of our tissue culture study<sup>10</sup> indicated that when PGF<sub>2α</sub> and PGE<sub>2</sub> were added to myofibroblast cell cultures they induced significant contractile responses. Specifically, in culture, PGF<sub>2α</sub>

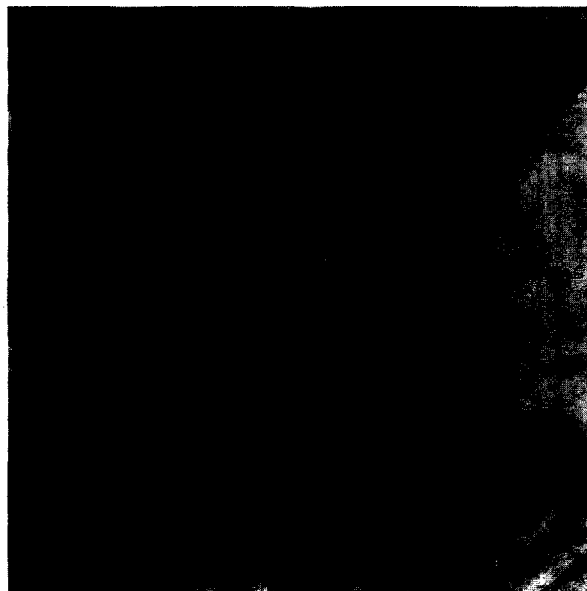
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**Fig. 1.** Electron micrograph of a myofibroblast from a patient with Dupuytren's disease. Prepared by immunohistochemistry for  $\text{PGF}_{2\alpha}$ . A fine grain reaction precipitate appears at the cell surface and within the cytoplasm, especially in association with myofilaments (*MF*). Rough endoplasmic reticulum (*ER*), the cell's nucleus (*N*), and collagen fibrils (*C*) are shown. This precipitation reaction is also representative of that observed for  $\text{PGE}_2$ . ( $\times 40,000$ .)

caused myofibroblasts to contract and  $\text{PGE}_2$  caused the cells to relax after previous constriction.

This study was undertaken to investigate if  $\text{PGF}_{2\alpha}$  and  $\text{PGE}_2$  were associated, *in vivo*, with myofibroblasts in Dupuytren's disease. Specific antibodies to each of these prostaglandins were used in immunocytochemical and radioimmunoassay studies. The latter technique was used to quantitate amounts of both  $\text{PGF}_{2\alpha}$  and  $\text{PGE}_2$  in patient's affected palmar fascia and in plasma. Such studies may provide insight into the biologic approach for treatment of Dupuytren's disease.

### Materials and methods

Nodules were obtained from 37 patients with Dupuytren's disease who were treated by partial fasciectomy. There were 28 men and nine women. The mean age was 56.2 years. Control samples of palmar fascia were obtained from 19 patients having hand surgery for carpal tunnel releases. There were seven men and 12 women. The mean age was 53.4 years. Ten milliliters of venous blood was collected from all patients and the plasma was recovered by centrifugation. This study was approved by an institutional review board that consisted of two separate committees. Informed, written consent

was obtained from all patients before obtaining tissue and blood for research use.

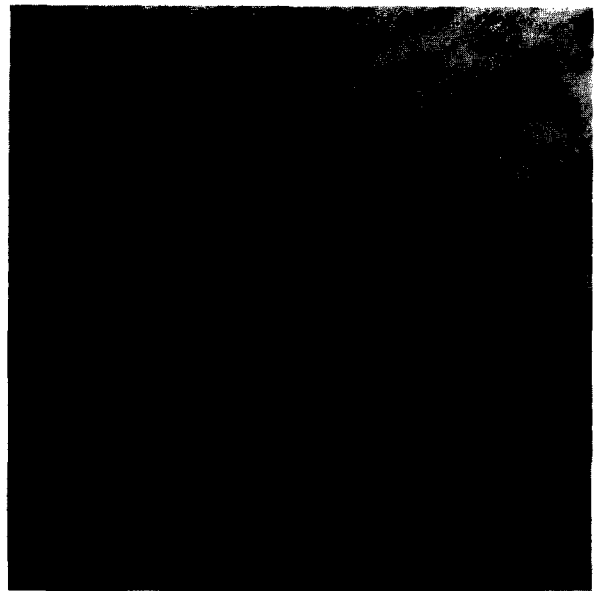
Dupuytren's nodules were identified at operation by palpation and by visualization using optical loupes with a  $3\frac{1}{2} \times$  magnification.

Excised nodules and control palmar fascia were sharply transected at their midpoints. One half of the nodules and control palmar fascia samples were immediately fixed in 0.15% glutaraldehyde, 4% paraformaldehyde-periodate-lysine fixative overnight. Samples were sectioned transversely at their midpoints on a vibratome at 200 to 300  $\mu\text{m}$ . This ensured that representative midnodular and midcontrol fascial samples were collected. For ultrastructural immunocytochemistry, sections were preincubated in 10% swine serum followed by incubation in  $\text{PGE}_2$  on  $\text{PGF}_{2\alpha}$  anti-rabbit antibody, 1:100 at 4° C for 12 hours. After a buffer rinse, sections were incubated in swine anti-rabbit IgG, 1:50 for 1 hour at 4° C followed by rabbit peroxidase antiperoxidase, 1:50 and reaction in 2.5% diaminobenzidine. Sections were then fixed in 1%  $\text{OsO}_4$  and prepared for transmission electron microscopy. Antibody specificity was controlled by adsorbing PG antibody to its identical antigen (100  $\mu\text{g}$  antigen/ml antiserum) and using this as a primary incubation medium. Incubations in primary antibody and secondary swine anti-rabbit IgG were also omitted as controls.

Radioimmunoassay (RIA) was performed to quantitate  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  concentrations in patient tissue samples and in plasma. The remaining half of the nodule and fascia samples, as well as all plasma samples, were immediately frozen after collection at  $-70^\circ\text{C}$ . The RIA measured the competitive binding of radiolabeled prostaglandin and unlabeled prostaglandin with antibody to prostaglandin. Tissue samples were homogenized in TRIS buffer, pH 7.2 and digested with collagenase (Form III, Advance Biofactures Co. Lynbrook, N.Y.) for 20 hours at room temperature, lyophilized, and extracted with petroleum ether. Plasma samples were also ether extracted. Radioimmunoassay was carried out with goat anti-rabbit  $\text{PGF}_{2\alpha}$  with a  $^3\text{H}$  label (Travenol). For  $\text{PGE}_2$  goat anti-rabbit  $^{125}\text{I}$  label was used (New England Nuclear, Boston, Mass.). The nonradioactive prostaglandins from the Dupuytren's and control extracts competed with a serially diluted but constant amount of labeled tracer for binding sites on the prostaglandin antibody. The antibody was held at a limited concentration. Therefore, the amount of tracer that bound to the antibody was inversely proportional to the amount of nonradioactive prostaglandin in the assay tube. Antibody-bound prostaglandin was separated from free prostaglandin by precipitation with a



**Fig. 2.** Electron micrograph of a fibroblast from a control patient. Prepared by immunohistochemistry for  $\text{PGF}_{2\alpha}$ . This photo is also representative of immunohistochemistry in control fibroblasts for  $\text{PGE}_2$ . Collagen fibrils (C) appear at the top. A slight reaction precipitate (arrows) appears at the cell surface. The fibroblast nucleus (N) and nucleolus (NU) occupy a large portion of the cell's mass. ( $\times 40,000$ .)



**Fig. 3.** Electron micrograph of a myofibroblast from a patient with Dupuytren's disease. Prepared as an antigen-antibody adsorption control for  $\text{PGF}_{2\alpha}$ . This photo is also representative of adsorption control results for  $\text{PGE}_2$ . A myofibroblast is shown surrounded by collagen fibrils (C). The nucleus (N), myofilaments (MF), and free ribosomes (R) are prominent. There is a negative immunohistochemical reaction for  $\text{PGF}_{2\alpha}$ . ( $\times 40,000$ .)

second antibody. The bound radioactivity was measured in a scintillation counter. A standard curve for  $\text{PGE}_2$  was established ranging from 0.1 to 1000 picograms. A standard curve for  $\text{PGF}_{2\alpha}$  was established ranging from 0.2 to 1000 pg. Prostaglandin values were interpolated from standard curves as picograms per milligram in tissue and picograms per milliliter in plasma from the standard curves.

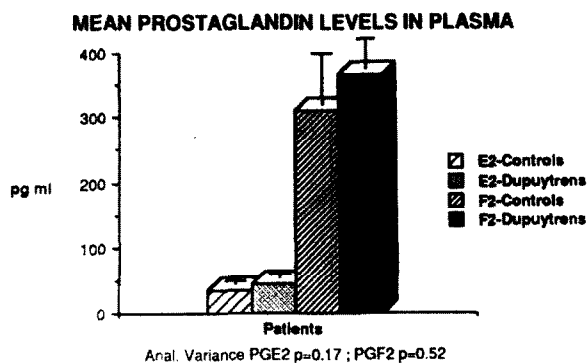
## Results

Myofibroblasts were identified by electron microscopy in all nodular samples from patients with Dupuytren's disease. These cells exhibited characteristic ultrastructural features including indented nuclei, bundles of cytoplasmic filaments aligned with the long axis of the cell, a well-developed system of rough endoplasmic reticulum, extensive distribution of free ribosomes, and junctional attachment membrane complexes with hemidesmosomes. Ultrastructural immunocytochemistry revealed that a positive reaction precipitate for  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  was present in all patient samples in association with myofibroblasts. The precipitate was present in association with the myofibroblast cell membrane and widely dispersed intracellularly (Fig. 1).

Fibroblasts were identified by electron microscopy in all samples of control palmar fascia. Fibroblasts had typical spindle shape, with scant cytoplasm and a large nucleus. Immunocytochemistry showed that  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  were present in association with fibroblasts but only as a slight reaction precipitate associated with the fibroblast cell membrane (Fig. 2).

Adsorption controls of each prostaglandin with its corresponding antibody and omission of the primary and secondary IgG incubations produced a negative precipitate reaction confirming specificity of the procedure (Fig. 3).

Radioimmunoassay of PGs in plasma revealed mean increases of 23%  $\text{PGE}_2$  and 16%  $\text{PGF}_{2\alpha}$  in patients with Dupuytren's disease when compared with controls (Fig. 4). However, after analysis of variance for significant differences between the sample means, it was determined that increases of  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  in the plasma of patients with Dupuytren's disease were not significant ( $p = 0.17 \text{ PGE}_2$ ;  $p = 0.52 \text{ PGF}_{2\alpha}$ ). In nodules, radioimmunoassay showed mean increases of 40%  $\text{PGE}_2$  and 55%  $\text{PGF}_{2\alpha}$  in patients with Dupuytren's disease when compared with control fascia (Fig. 5). Mean



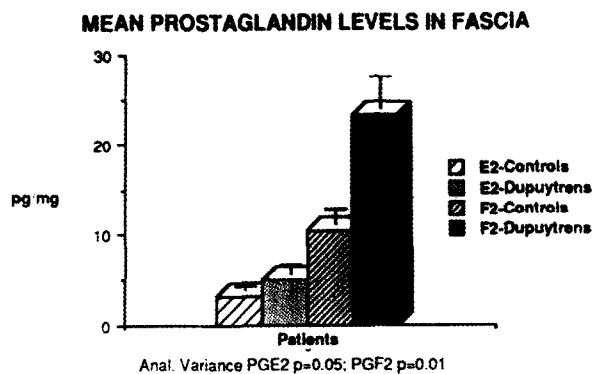
**Fig. 4.** Mean prostaglandin levels in plasma of patients with Dupuytren's disease and control patients measured as picograms per milliliter by radioimmunoassay. Both PGE<sub>2</sub> and PGF<sub>2α</sub> levels were increased in plasma of patients with Dupuytren's disease when compared with control patients. However, these increases were *not* significant after analysis of variance of sample means.

increases, after analysis of variance for significance between sample means in Dupuytren's nodules *were significant* when compared with control palmar fascia ( $p = 0.05$  PGE<sub>2</sub>;  $p = 0.01$  PGF<sub>2α</sub>).

#### Discussion

It has become increasingly apparent that myofibroblasts in Dupuytren's disease are contractile cells. *In vitro* investigations have demonstrated that a variety of substances can induce myofibroblasts to contract.<sup>7</sup> The debate linking myofibroblast contraction to the flexion deformities of Dupuytren's disease has routinely centered on the lack of an apparent physical connection from cell to cell and cell to collagen. The recent finding that fibronectin and filaments in the extracellular matrix connect filament bundles within myofibroblasts to collagen fascicles in the extracellular space and myofibroblasts to each other<sup>9</sup> may provide an explanation consistent with the translation of contractile forces to collagen. On the basis of these studies, the myofibroblast has been described as playing an important role in the contraction of connective tissue, a process that may be beneficial in such situations as wound closure, but harmful in other situations such as the fixed flexion contractures in Dupuytren's disease. Thus, endogenous factors that may influence myofibroblast contractility in affected palmar fascia may hold significance in the cause, pathogenesis, and treatment of Dupuytren's disease.

Our previous study has shown that in culture the vasoactive prostaglandins PGF<sub>2α</sub> and PGE<sub>2</sub> cause myofibroblasts from Dupuytren's disease to contract and



**Fig. 5.** Mean prostaglandin levels in palmar fascia of patients with Dupuytren's disease and control patients measured as picograms per milligram by radioimmunoassay. Both PGE<sub>2</sub> and PGF<sub>2α</sub> levels were increased in Dupuytren's fascia when compared with controls. Proportionately, there was a larger increase of PGF<sub>2α</sub>. Increases were significant after analysis of variance of sample means.

relax, respectively. The present study has immunohistochemically identified prostaglandins E<sub>2</sub> and F<sub>2α</sub> in association with myofibroblasts of Dupuytren's disease. In addition, quantitation by radioimmunoassay has shown increased levels of these prostaglandins, especially F<sub>2α</sub>, in affected palmar fascia.

Prostaglandins (PG) are polyunsaturated, hydroxylated, long-chain fatty acids discovered in the early 1930s. Prostaglandins generally are not stored within cells to any great extent.<sup>11, 12</sup> Stimulation for their release requires substrate precursors, such as free fatty acid. Polyunsaturated fatty acids, such as arachidonic acid, are the principal substrates for PG formation. The release of these polyunsaturated fatty acids is facilitated by the hydrolytic action of a class of membrane associated enzymes known as phospholipases.<sup>11, 12</sup> Arachidonic acid can also interact with the enzyme prostaglandin synthetase generating prostaglandin intermediates, known as endoperoxides.<sup>11, 12</sup> By a complex series of enzymatic reactions, endoperoxides are degraded to form prostaglandins, thromboxanes, or prostacyclins.<sup>11, 12</sup>

The specific prostaglandins demonstrated in this study are well known to influence smooth muscle, especially, vascular contractility.<sup>11, 12</sup> We have previously demonstrated that PGE<sub>2</sub> and PGF<sub>2α</sub> in culture influence contractility on myofibroblasts from Dupuytren's disease.<sup>6</sup> These prostaglandins may exert similar contractile responses on myofibroblasts in Dupuytren's disease to contribute to collagen deformation and ultimately to joint contractures. The significant increase in PGF<sub>2α</sub> in Dupuytren's nodules would appear to indicate that this

substance may be locally available. Its availability may derive from the microcirculation, since nodules are known to be highly vascular.<sup>13</sup> Also, we have demonstrated circulating levels of prostaglandins in plasma. In addition,  $\text{PGF}_{2\alpha}$  may be available by its fatty acid precursor, arachidonic acid, as Dupuytren's nodules are also known to have a high lipid content.<sup>14</sup>

Prostaglandin  $\text{E}_2$ , presumably, may be available from the same sources within affected nodules. If this is so, it may be argued that  $\text{PGE}_2$  may exert its physiologic relaxation effects. However, in this study, radioimmunoassay quantitation in Dupuytren's nodules showed that although  $\text{PGE}_2$  was increased, proportionately, there was significantly more  $\text{PGF}_{2\alpha}$  per milligram of fascia than  $\text{PGE}_2$ . These increased levels of prostaglandins in Dupuytren's nodules shown by radioimmunoassay could be caused by the increased cellularity of the nodular tissue. This presupposes that myofibroblasts are the source of the prostaglandins. As we have cited previously, the more likely source of prostaglandins is from the microcirculation and/or tissue fatty acid. Further, the literature lends support to the contention that cells in granuloma tissue (myofibroblasts) are targets and under the control of prostaglandins.<sup>15</sup>

To the practicing hand surgeon, the demonstration of prostaglandins in the nodules of Dupuytren's disease may seem an interesting but esoteric finding. In the practical sense, one might speculate that a basis for successful nonsurgical treatment for early or mild recurrent disease might be the inhibition of prostaglandin synthesis in the nodules of Dupuytren's disease. After completing this study, the authors enjoyed reading Dr. Ketchum's report in which he employed such a treatment. In a 16-year study of 60 patients with Dupuytren's disease, staged, short-term intranodular injections of triamcinolone in the *early stages* of Dupuytren's disease was shown to reduce or abolish palmar nodules, with subsequent return of hand function.<sup>16</sup> Subsequently, the same investigator has used intranodular triamcinolone injections in combination with contracture release and full-thickness skin grafts.<sup>17</sup> Among its effects, triamcinolone is an anti-inflammatory corticosteroid inhibitor of prostaglandin biosynthesis, which works by limiting the availability of substrate fatty acids. Thus, the present study may provide a foundation in basic science for the clinical application of prostaglandin inhibitors, especially in early or mild recurrent Dupuytren's disease.

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