Pharmacologic Regulation of Dupuytren's Fibroblast Contraction *In Vitro*

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Dupuytren's disease is associated with contraction of specialized fibroblasts present in the diseased palmar fascia. Pharmacologic agents were evaluated for their ability to promote or inhibit contraction of Dupuytren's fibroblasts *in vitro* using a collagen lattice contraction assay. In the first part of the study, lysophosphatidic acid (LPA), serotonin, angiotensin II, and prostaglandin $F_{2\alpha}$ were tested for their ability to promote Dupuytren's fibroblast contraction. Lysophosphatidic acid was found to significantly promote Dupuytren's fibroblast contraction as compared with controls. This response to LPA is dose dependent, with a half-maximal response of 0.07 μM. Angiotensin II, serotonin, and prostaglandin $F_{2\alpha}$ at 1 mM, induced a significant amount of contraction as compared to controls, but the amount of contraction was at least six times less than that observed for LPA. In the second part of the study, prostaglandins E_1 and E_2 or the calcium channel blockers nifedipine and verapamil were tested for their ability to inhibit LPA-promoted contraction. It was found that both types of inhibitors partially block LPA-promoted contraction of Dupuytren's fibroblasts. The effect of the various pharmacologic agents on normal palmar fibroblasts was not evaluated. The focus of this study was to examine the regulation of contraction of Dupuytren's fibroblasts. This study demonstrates that LPA is a potent agonist of Dupuytren's fibroblast contraction and that this contraction can be inhibited by specific pharmacologic agents. These findings provide a rational basis for investigating further the clinical use of the calcium channel blockers nifedipine or verapamil and prostaglandins E_1 and E_2 to control Dupuytren's disease and possibly other fibrotic conditions. (J Hand Surg 1996;21A:1065–1070.)

Dupuytren's diseased tissue contains specialized fibroblasts that have acquired contractile properties and have been termed *myofibroblasts*. These cells have large intracellular bundles of actin microfila-

ments, wrinkled nuclei, extracellular fibronectin fibrils at their surfaces, and transmembrane associations termed the *fibronexus*, linking actin and fibronectin fibrils.[1-6] Based upon the presence of these cells during active contraction of the Dupuytren's fascia and their histologic characteristics, it has been proposed that they generated the forces responsible for palmar fascia contracture.[1-6]

We have developed an *in vitro* collagen lattice contraction assay and showed that cultured Dupuytren's fibroblasts formed bundles of actin microfilaments and fibronectin fibrils identical to Dupuytren's fibroblasts *in vivo*.[7-9] These Dupuytren's fibroblasts can generate contractile force as demonstrated by their ability to rapidly contract a collagen lattice after release from its points of stabilization. We have previ-

ously used the same model to demonstrate that Dupuytren's fibroblast contraction is promoted by unknown factors in serum.[7]

The purpose of this study was to identify specific agents that have the potential to promote or inhibit the contraction of Dupuytren's fibroblasts. Understanding and manipulating the cellular mechanisms of Dupuytren's fibroblast contraction will facilitate the design of therapeutic agents and optimize strategies for controlling Dupuytren's disease and other fibrotic conditions.

Materials and Methods

Angiotensin II, serotonin, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and lysophosphatidic acid (LPA) were tested for their ability to promote Dupuytren's fibroblast contraction. These were used as agonists because they promote smooth-muscle contraction.[10-13] Lysophosphatidic acid can also stimulate the formation of bundles of actin microfilaments in cultured fibroblasts,[14] promote the retraction of neuronal cells,[15] and is present in serum at concentrations of 1–5 μM.[16] Angiotensin and serotonin can promote contraction of isolated strips of granulation tissue containing myofibroblasts,[17,18] and $PGF_{2\alpha}$ has been reported to promote contraction of Dupuytren's fibroblasts at concentrations of 0.5–2 mM.[19]

Nifedipine, verapamil, and prostaglandins E_1 (PGE_1) and E_2 (PGE_2) were used as antagonists and tested for their ability to inhibit Dupuytren's fibroblast contraction. Nifedipine and verapamil were selected because they inhibit the contraction of smooth muscle in response to agonists, presumably by blocking the intracellular rise in Ca^{++}.[20] PGE_1 and PGE_2 were selected because they can inhibit the contraction of smooth muscle by activating adenylate cyclase and elevating cyclic adenosine monophosphate (cAMP) levels.[12] Additionally, PGE_2 has been reported to inhibit Dupuytren's fibroblast contraction at concentrations of 0.5–2 mM.[19]

Cell Culture

Dupuytren's fibroblast explant cultures were obtained from patients undergoing surgery for Dupuytren's contracture as previously described.[8,9] The Dupuytren's nodular tissue was dissected from the surrounding cord and normal-appearing palmar fascia. Pieces of nodule were placed into 60-mm culture dishes (Falcon, Oxnard, CA). They were cultured in complete media containing M-199 (GIBCO Laboratories, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (Irvine Scientific, Santa Ana, CA), 2 mM glutamine, and 1% antibiotic-antimycotic solution. Cultured Dupuytren's fibroblasts were harvested using trypsin-ethylenediaminetetraacetic acid (EDTA) (GIBCO) and subcultured in 75-mm^2 tissue culture flasks. Three cell strains were used in these experiments and subcultured fewer than 10 passages.
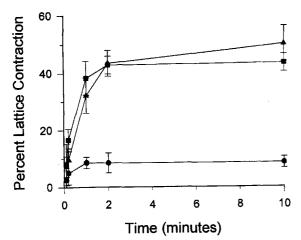
Collagen Lattice Contraction Assay

Cells from each strain were cultured within stabilized type I collagen lattices as previously described.[7-9] Acetic acid solubilized rat tail tendon type I collagen (Upstate Biotechnology, Inc., Lake Placid, NY) was brought to physiologic pH and ionic strength at 4°C and mixed with suspended fibroblasts.[7-9] The final collagen concentration was 0.65 mg/mL and the cell concentration was 1.25×10^5 cells/mL. A 250-μL drop of the collagen–cell suspension was placed on a 35-mm plastic tissue culture dish. After the collagen lattice was incubated for 1 hour at 37°C, to allow for gelling of the collagen, 1.5 mL of complete medium was placed over the collagen lattice. Care was taken not to detach the lattices from the underlying plastic substratum. The stabilized lattices were incubated for 5 days in complete media.

After 5 days in culture, Dupuytren's fibroblast contraction was assayed. Lattices were washed twice with medium-lacking FBS for 5 minutes. Agonists were added to the lattice in the absence of FBS and the stabilized lattices were mechanically released from the underlying substratum by gently pipetting media at the collagen–plastic interface.[7-9] Serum-deprived lattices in the absence of other factors were used as controls, three for each strain. Antagonists were added to serum-deprived lattices, followed by addition of LPA and mechanical release of the lattice from the underlying substratum. Rapid contraction was analyzed by measuring the diameter of the lattice before and at various times after release, using a Nikon SMZ-1 stereoscope (Nikon Inc. Melville, NY). Lattice diameters were normalized, owing to variations in the initial diameter of the lattices, which ranged from 14 to 16 mm. The relative lattice diameter was obtained by dividing the diameter of the collagen lattice at each time point by the initial diameter of the lattice. The percent contraction was obtained by subtracting the relative lattice diameter from 1. In each experiment, all factors were tested using triplicate lattices, and each experiment was repeated three times. Data are expressed as the mean ± SD. Statistical analysis

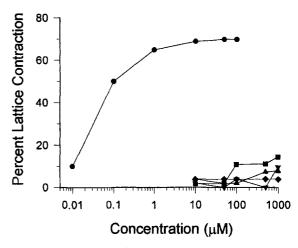was done using an independent group Student's *t*-test (Crunch Software Corp., Oakland, CA).

Results

Agonists

Lysophosphatidic acid (1 μM) promoted rapid collagen lattice contraction of Dupuytren's fibroblasts in a time-dependent manner. The result was a significant increase in collagen lattice contraction over serum-deprived (no agonist) collagen lattices, 43.5 ± 3.1% as compared to 8.5 ± 2.0% 10 minutes after release (p < .01). The amount of contraction promoted by 1 μM LPA was similar to that observed for 10% FBS (Fig. 1). This response to LPA was dose dependent, with a half-maximal response of approximately 0.07 μM (Fig. 2). Angiotensin II required a concentration of 100 μM to promote contraction of Dupuytren's fibroblasts, while serotonin and $PGF_{2\alpha}$ required a concentration of 500 μM and 1000 μM, respectively (Fig. 2). Although angiotensin II, serotonin, and $PGF_{2\alpha}$ could all significantly promote contraction of Dupuytren's fibroblasts over no agonist (p < .05), the amount of contraction promoted was at least six times less than that promoted by LPA (Fig. 2). The half-maximal responses were 75 μM, 250 μM, and 750 μM for angiotensin II, serotonin, and $PGF_{2\alpha}$ respectively. Therefore, LPA is at least 1,000 times more active in promoting contraction than angiotensin II, serotonin, or $PGF_{2\alpha}$.
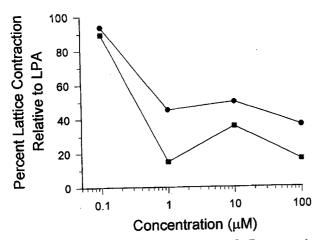

Figure 2. Dose-dependent increase in Dupuytren's fibroblast contraction in response to lysophosphatidic acid (LPA), angiotensin II, serotonin, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or no agonist. The percent collagen lattice contraction that occurred 10 minutes after release increased dramatically with increasing concentrations of LPA (●). A small but significant amount of collagen lattice contraction occurred with increasing concentrations of angiotensin II (■), serotonin (▲), and $PGF_{2\alpha}$ (▼), compared to no agonist (♦) (p < .05), although much less than with LPA. This graph represents one experiment with triplicate lattices. Standard deviations are shown.

Antagonists

Serum-deprived collagen lattices were incubated for 10 minutes with either PGE_1 or PGE_2; LPA was then added and contraction was measured. PGE_1 and PGE_2 inhibited LPA-promoted (1 μM) contraction in a dose-dependent manner (Fig. 3). PGE_1 and PGE_2 significantly inhibited LPA-promoted contraction at concentrations greater than 1 μM (p < .01), but PGE_2 inhibited contraction to a greater extent than did PGE_1. The smooth-muscle inhibitors nifedipine and verapamil were tested in the same manner by incubating the lattices for 30 minutes with either agent, adding LPA (1 μM), and measuring contraction. Nifedipine and verapamil both inhibited LPA-promoted contraction in a dose-dependent manner and to a similar extent (Fig. 4). Nifedipine and verapamil significantly inhibited LPA-promoted contraction at concentrations greater that 100 μM (p < .01).

Discussion

Contractile fibroblasts or myofibroblasts are encountered in many fibrotic conditions, including Dupuytren's disease.[21] These cells from diverse pathologic conditions share the same morphologic charac-

Figure 1. Lysophosphatidic acid (LPA) promoted contraction of Dupuytren's fibroblasts. The percent contraction of collagen lattices containing Dupuytren's fibroblasts increased over time when released in the presence of 1 μM LPA (▲) or 10% fetal bovine serum (FBS) (■). Little contraction occurred in the absence of FBS or LPA (●). This graph represents one experiment with triplicate lattices. Standard deviations are shown.

Figure 3. Dose-dependent response of Dupuytren's fibroblasts to prostaglandins E_1 and E_2 (PGE_1 and PGE_2). The percent collagen lattice contraction in response to lysophosphatidic acid (LPA) (1 μM) at 10 minutes after release was normalized to 100%. PGE_1 (■) and PGE_2 (●) inhibited LPA-promoted contraction in a dose-dependent manner. This graph represents one experiment with triplicate lattices.

teristics, and at one stage in their life cycle, they actively contract, resulting in tissue contracture.[21] We have previously demonstrated that Dupuytren's fibroblasts can contract collagen lattices only in the presence of FBS.[7-9] This suggests that unknown fac-
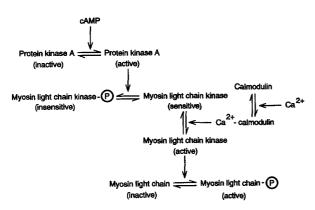

Figure 4. Dose-dependent response of Dupuytren's fibroblasts to nifedipine and verapamil. The percent collagen lattice contraction in response to lysophosphatidic acid (LPA) (1 μM) at 10 minutes after release was normalized to 100%. Nifedipine (●) and verapamil (■) inhibited LPA-promoted contraction in a dose-dependent manner. This graph represents one experiment with triplicate lattices.

tors present in FBS are necessary for the promotion of Dupuytren's fibroblast lattice contraction.

In this study, we identified factors that promote and inhibit the contraction of Dupuytren's fibroblasts *in vitro*. We found that LPA promotes the contraction of Dupuytren's fibroblasts, whereas nifedipine, verapamil, PGE_1, and PGE_2 inhibit LPA-promoted contraction. Lysophosphatidic acid may be a primary agonist in FBS that promotes contraction of Dupuytren's fibroblasts. This hypothesis is based on a number of observations. Lysophosphatidic acid is a phospholipid that is released by platelets into FBS during preparation at concentrations of 1–5 μM.[16] This concentration is in the range of purified LPA required to promote Dupuytren's fibroblast contraction. We have previously demonstrated that the active agonist for fibroblast contraction in FBS is a lipid-type molecule based on fractionation of serum.[22] Additionally, boiling FBS for 10 minutes does not destroy its contraction-promoting activity, suggesting that the prominent factor is not a large polypeptide.[22] Preliminary results from our laboratory suggest that treatment of whole-blood serum with phospholipase B, which breaks down LPA, blocks serum-promoted contraction (Parizi et al., unpublished observations, 1995). Lysophosphatidic acid is a crucial intermediate in *de novo* lipid biosynthesis that is produced in many cells.[16] In a manner similar to that of prostaglandins, LPA may be released into the extracellular environment upon appropriate stimulation. Once released, it can act on adjacent cells in a paracrine manner or on itself in an autocrine manner. Dupuytren's disease patients have significantly higher fasting serum cholesterol and triglyceride levels than normal.[23] Alcoholism is associated with elevated serum triglyceride levels[24] and phenobarbitone is known to increase cholesterol metabolism,[25] both conditions known to be associated with Dupuytren's disease.[26,27] Additionally, PGE levels appear to be decreased under these conditions.[28,29] Increased LPA and decreased PGE acting in concert may contribute to the contraction of Dupuytren's palmar fascia.[19]

Lysophosphatidic acid may promote Dupuytren's fibroblast contraction by activation of specific secondary messenger cascades through binding to its cell membrane receptor,[30] decreasing levels of cAMP[31] and increasing levels of Ca^{++}.[32] Intracellular cAMP and Ca^{++} levels may regulate the activity of myosin light-chain kinase, an enzyme that phosphorylates myosin light chain (Fig. 5). Myosin light-chain phos-

Figure 5. Intracellular signaling cascade regulating Dupuytren's fibroblast contraction.

phorylation is required for the myosin–actin interaction and the generation of contractile force.[33,34] PGE_1 and PGE_2 increase intracellular levels of cAMP and effect smooth-muscle contraction in the namomolar to micromolar range, similar to the concentrations used in this study.[12] These agents may inhibit the ability of LPA to decrease cAMP, which interferes with phosphorylation of myosin light chain, with subsequent decreased contraction. Nifedipine and verapamil inhibit the intracellular rise in Ca^{++} and affect smooth muscle in the namomolar to micromolar range,[20] suggesting that Dupuytren's fibroblasts are less sensitive than smooth muscle to these antagonists.

Hurst and co-workers demonstrated that PGE_2 causes relaxation of cultured Dupuytren's fibroblasts in the presence of FBS.[19] Our results are consistent with their findings, except that we obtained inhibition at 1 µM for PGE_2, as opposed to 0.5–5 micromolar. Additionally, we demonstrated that PGE_1 inhibits Dupuytren's fibroblast contraction. Hurst and co-workers also demonstrated that 0.5–5 mM $PGF_{2\alpha}$ could induce contraction of cultured Dupuytren's fibroblasts in the presence of FBS.[19] In our study, $PGF_{2\alpha}$ promoted contraction of Dupuytren's fibroblasts at a similar concentration, but the amount of contraction was dramatically less than that observed for LPA. LPA was over 1,000 times more potent, suggesting that it is a much more important agonist for Dupuytren's fibroblast contraction than $PGF_{2\alpha}$.

The lack of Dupuytren's fibroblast contraction upon exposure to angiotensin and serotonin in our study is inconsistent with Gabbiani and coworkers' results, which showed these agents could promote the contraction of granulation tissue strips.[17,18] Granulation tissue contains a heterogeneous popula-

tion of cells and a variety of factors. Angiotensin and serotonin could be acting synergistically, with other factors in the granulation tissue to promote contraction, or indirectly by affecting different cell types, resulting in the release of a contractile substances, such as LPA.

We believe that the use of Dupuytren's versus normal fibroblasts as controls in this study is irrelevant. We have previously demonstrated that normal palmar fibroblasts acquire characteristics similar to those of Dupuytren's fibroblasts when cultured within collagen lattices.[7,8] Not surprisingly, preliminary results have demonstrated that normal palmar fibroblasts respond similarly to the agonists and antagonists examined in this study (Parizi et al., unpublished observations, 1995). Numerous investigators[1,3,5,7,9,18] proposed that under the appropriate conditions, normal palmar fibroblasts can modulate into myofibroblasts.

Recently, Lee and co-workers[35] showed that direct injection of verapamil into hypertrophic burn scars results in scar size reduction. The reduction in scarring may be the result of decreased tension in the wound generated by the myofibroblasts in response to verapamil and subsequent remodeling of the tissue. Our study demonstrates that nifedipine or verapamil can inhibit the contraction of Dupuytren's fibroblasts. These agents should be investigated further for possible clinical use to control Dupuytren's contracture. Understanding the cellular mechanisms responsible for contraction of Dupuytren's fibroblasts may improve methods of management of palmar fascia contraction and may lead to the development of therapeutic agents to control not only Dupuytren's disease but other fibrotic conditions as well.

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