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Tamoxifen Decreases Fibroblast Function and Downregulates $TGF_{\beta 2}$ in Dupuytren's Affected Palmar Fascia[1]

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Background. **Dupuytren's contracture is a fibroproliferative disorder that is associated with increased collagen deposition. Isoforms of transforming growth factor beta (TGF_{β}), normally $TGF_{\beta 1}$ and $TGF_{\beta 2}$, are involved in the progressive fibrosis of Dupuytren's disease. It has been suggested that downregulation of TGF_{β} may be useful in the treatment of the condition. Tamoxifen, a synthetic nonsteroidal antiestrogen, is known to modulate the production of TGF_{β}. This study examined the role of tamoxifen in decreasing fibroblast function and downregulating $TGF_{\beta 2}$.**

Methods. **Primary cultures of fibroblasts were obtained from Dupuytren's affected fascia and carpal tunnel affected fascia as a control. Collagen lattices were prepared and populated with the fibroblasts. The fibroblast-populated collagen lattices (FPCL) were then measured for contraction every 24 h for 5 days. Supernatant was obtained from the culture medium following completion of the FPCL portion of the experiment and used for a $TGF_{\beta 2}$ immunoassay.**

Results. **Dupuytren's affected fibroblasts contracted the FPCLs significantly more than carpal tunnel control fibroblasts. Treating the fibroblasts with tamoxifen caused a decreased contraction rate in both Dupuytren's affected fibroblasts and carpal tunnel controls. There was increased $TGF_{\beta 2}$ expression in the Dupuytren's affected fascia group compared to the carpal tunnel control group. Tamoxifen decreased $TGF_{\beta 2}$ expression in Dupuytren's affected fascia group but not in the carpal tunnel control group.**

Conclusion. **TGF_{β} appears to be the key cytokine in the fibrogenic nature of Dupuytren's disease. Tamoxifen treatment has been demonstrated to decrease the function of fibroblasts derived from Dupuytren's affected fascia and downregulated $TGF_{\beta 2}$ production in these same fibroblasts. These data suggest a method to manipulate and control Dupuytren's contracture in the clinical setting.** © 2002 Elsevier Science (USA)

Key Words: **Dupuytren's contracture; tamoxifen; TGF_{β}; fibroblast.**

INTRODUCTION

Dupuytren's contracture is a fibroproliferative disorder characterized by progressive, irreversible flexion of one or more digits that was initially described in 1832 [1–3]. While its etiology remains unclear, Dupuytren's contracture is known to result from changes produced in the dermis and palmar fascia [2]. These changes include proliferation of myofibroblasts, which were first defined as the dominant cell type associated with the formation of the pathognomonic nodule of the palmar fascia [4–6]. Dupuytren's contracture has also been associated with variable deposition of mature collagen fibers and enhanced synthesis of the extracellular matrix [2, 7].

Growth factors such as basic fibroblast growth factor and isoforms of transforming growth factor beta (TGF_{β}) are potent modulators of fibroblast and myofibroblast proliferation and differentiation. These cytokines have been shown to affect collagen synthesis as well and have been implicated in the pathobiology of proliferative scarring such as keloid formation and Dupuytren's disease [7–14].

Recent work produced in this laboratory suggests that TGF_{β} (especially $TGF_{\beta 2}$) may be the key cytokine involved in the progressive fibrosis of Dupuytren's disease [15]. It was suggested that compounds that abrogate, neutralize, or downregulate the isoforms $TGF_{\beta 1}$

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and/or $TGF_{\beta2}$ may be useful in the treatment of the condition.

Tamoxifen, used mainly in the treatment of breast cancer, is a synthetic nonsteroidal antiestrogen that may also be effective in the treatment of abnormal proliferative healing disorders such as retroperitoneal fibrosis and desmoid tumors [16–19]. One of the demonstrated effects of tamoxifen is modulation of the production of growth factors such as TGF_{α}, TGF_{β}, epidermal growth factor, and insulin-like growth factor (IGF) [16, 20–23]. In addition, tamoxifen has been demonstrated to decrease proliferation of both normal dermal and keloid fibroblasts as well as decrease the synthesis of collagen by keloid fibroblasts [16, 24].

Previous studies performed with human fibroblasts have demonstrated a decrease in fibroblast function in fibroblast-populated collagen lattices (FPCL) with tamoxifen treatment. Since Dupuytren's disease is a fibroproliferative process with increased $TGF_{\beta2}$ production, we examined the role of tamoxifen at decreasing fibroblast function and downregulating $TGF_{\beta2}$.

METHODS

Preparation of the fibroblast cultures. Primary cultures of fibroblasts were obtained from Dupuytren's affected fascia and carpal tunnel affected fascia [2, 8, 25]. Samples of palmar fascia were obtained at surgical release from six patients who had fasciectomy for treatment of Dupuytren's disease. Control palmar fascia was obtained from six patients who had hand surgery for carpal tunnel release. All patients were undergoing elective excision independent of this study. Primary cultures of fibroblasts from the surgical specimens were then established. Cells from passages 3 to 5 were used for experiments. The specimens were rinsed in 10 ml of calcium- and magnesium-free Dulbecco's phosphate-buffered saline (Sigma Chemical Co., St. Louis, MO) supplemented with gentamicin (20 mg/ml) for 30 min at room temperature. A second antibiotic rinse using 10,000 u/ml penicillin G, 25 μg/ml amphotericin B, and 10,000 μg/ml streptomycin sulfate solution (Gibco BRL, Grand Island, NY) was performed for 10 min. Each specimen was then cut into four pieces of equal dimensions and placed on the surface of a sterile 100-mm culture dish. The specimens were incubated with no additional culture medium for 15 min at 37°C. A 10-ml aliquot of Dulbecco's modified Eagle's medium (Gibco BRL) was carefully and slowly added to the culture dish which was then incubated at 37°C in 5% CO_2. The cells were subcultured until 80% confluence was obtained by removing the medium and tissue fragments from the culture dish with calcium- and magnesium-free Dulbecco's phosphate-buffered saline solution (Gibco). Trypsin–ethylenediaminetetraacetic acid (0.25%) (Gibco) was added and the cultures were incubated at 37°C for 15 min. A 15-ml aliquot of soybean trypsin inhibitor (Sigma) was added. The cultures were centrifuged at 1000 *g* for 10 min. The supernatant was decanted and the cell pellets were resuspended in 5 ml Dulbecco's modified Eagle's medium. This rinse/wash and 10-min centrifuge was repeated three times. The cells were counted with a hemacytometer and trypan blue was used to determine cell viability. The cell density was adjusted to 5×10^5 cells/ml with Dulbecco's modified Eagle's medium.

Preparation of collagen lattices. The collagen lattices were prepared from type I rat tail collagen (acetic acid extracted) as recommended by the manufacturer (Upstate Biotechnology, Lake Placid, NY). Undiluted collagen (3.85 mg/ml) was placed in 35-mm culture dishes (Falcon 1008) and 1 ml was evenly spread. The dishes were placed in an ammonia vapor chamber for 3 min to solidify. Sterile distilled water (5 ml) was added to the culture dishes, allowed to stand for 1 h, and then aspirated. This was repeated four times to remove excess ammonia and the collagen gel lattices were then incubated for 24 h at 4°C. Phosphate-buffered saline with 1.0% serum was added to replace the final aspirate. An 18-gauge needle was used to detach the collagen gel lattices from the surface of the culture dishes so that they were loose and suspended in the saline. A total of 72 collagen lattices were prepared to allow triplicate measurement from each specimen based on two treatment groups. To form the fibroblast-populated collagen lattices all saline was aspirated from the 35-mm culture dishes containing the collagen gel lattices. Two milliliters of 5×10^5 cells/ml was placed on the surface of each of the prefabricated collagen gel lattices. Tamoxifen (Sigma) was dissolved in PBS at a concentration of 8 μmol/ml and was added on day 0 to 18 of the FPCLs containing Dupuytren's fibroblasts and 18 of the FPCLs containing carpal tunnel fibroblasts. The 8 μmol/ml concentration was chosen because this dose has been shown not to be toxic to fibroblasts and to allow continued proliferation of fibroblasts [26]. This dose also allows fibroblast morphology to remain normal in collagen lattices [26].

Assay for gel contraction. The FPCLs were incubated at 37°C in a humidified atmosphere of 5% carbon dioxide. The amount of gel contraction was measured every 24 h for 5 days. Acetate overlays were used to trace the area of the gels. Gels were performed in triplicate for three cell lines established and measurements were then calculated using digital planimetry and Sigma Scan software (Jandel Scientific, Corte Madera, CA). Each collagen gel area measurement was converted to reflect the percentage of area remaining over time and subsequently the percentage of gel contraction. A one-way analysis of variance was used to determine significant differences among groups. When a difference was identified, Tukey's test (all pairwise multiple-comparison test) was used to delineate the differences. Sigma Stat statistical software (Jandel Scientific) was used for data analysis.

Immunoassay for $TGF_{\beta\text{-}2}$. The supernatant obtained from the culture medium following completion of the FPCL portion of the experiment was retained and used for a Quantikine human $TGF_{\beta2}$ immunoassay (R&D Systems, Minneapolis, MN). The supernatant was initially stored at −85° C after being mixed with 1 ml DMEM, 1 ml 1% fetal bovine serum, and 1 ml 1% penicillin/streptomycin. The samples were activated with 1 N HCl and incubated 10 min. Then 1.2 N NaOH/0.5 M Hepes and 0.8 ml calibrator diluent RD51 were added. After 2 h, the samples were assayed. During this time $TGF_{\beta2}$ standards were prepared. The assay procedure was as follows: 100 μl RD1-17 was added to each well then 100 μl standard or sample was added to each well. These were incubated for 2 h and then aspirated and washed three times. Next, 200 μl of conjugate was added to each well, incubated for 2 h, aspirated, and washed three times. A total of 200 μl of substrate solution was added to each well, the wells were incubated for 20 min, and then 50 μl of stop solution was added to each well. The wells were read upon completion of the immunoassay using a Microplate Manager 4.0 (Bio-Rad Laboratories, Inc., Hercules, CA) at a wavelength of 450 nm with correction of 540 nm. Once the standard curve was complete the unknown concentrations were determined. The program calculated mean absorbance (OD), standard deviation, and concentration. Each unknown concentration was then multiplied by a factor of 7.8 to correct for the dilution factor. A one-way analysis of variance was used to determine significant differences among groups. When a difference was identified Tukey's test (all pairwise multiple-comparison test) was used to delineate the differences. Sigma Stat statistical software (Jandel Scientific) was used for data analysis.

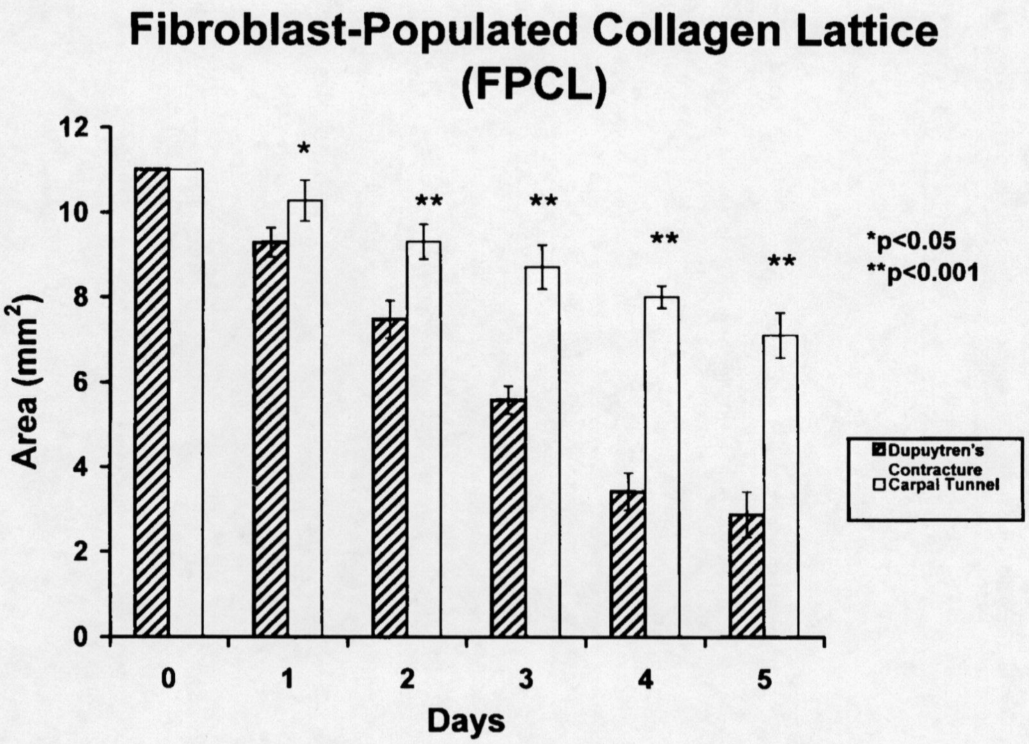

FIG. 1. Fibroblasts derived from Dupuytren's affected palmar fascia demonstrated significantly greater contraction over days 1–5 compared to fibroblasts derived from carpal tunnel (normal).

RESULTS

FPCL Contraction

When comparing the percentage of contraction over the 5-day study period, FPCLs populated with fibroblasts obtained from fascia affected by Dupuytren's disease contracted significantly more at days 1, 2, 3, 4, and 5 compared to the FPCLs populated with fibroblasts from the carpal tunnel control ($n = 18$, $P < 0.05$, ANOVA) (Fig. 1). FPCLs populated with fibroblasts obtained from Dupuytren's disease affected fascia that were treated with tamoxifen had significantly decreased contraction rates on days 1, 2, 3, 4, and 5 compared to untreated FPCLs ($n = 18$, $P < 0.05$, ANOVA) (Fig. 2). Fibroblasts obtained from patients with carpal tunnel (normal palmar fibroblasts) that were treated with tamoxifen did affect FPCL contraction on days 3, 4, and 5 when compared to untreated fibroblasts ($n = 18$, $P < 0.05$, ANOVA) (Fig. 3). However, the FPCL contraction was to a lesser degree than that of the FPCL containing fibroblasts obtained from Dupuytren's affected fascia.

$TGF_{\beta 2}$ Immunoassay

There was a significant increase in $TGF_{\beta 2}$ expression in the supernatant obtained from FPCLs populated with fibroblasts obtained from Dupuytren's affected fascia compared to the supernatant obtained from FPCLs populated with fibroblasts obtained from carpal tunnel affected fascia ($n = 18$, $P < 0.05$, ANOVA) (Fig. 4). Tamoxifen treatment of fibroblasts obtained from Dupuytren's affected fascia resulted in a significant downregulation of $TGF_{\beta 2}$ expression compared to untreated fibroblasts ($n = 18$, $P < 0.05$, ANOVA) (Fig. 5). Supernatant from FPCLs populated with fibroblasts obtained from carpal tunnel fascia (normal) that were treated with tamoxifen did not show any significant downregulation of $TGF_{\beta 2}$ compared to untreated fibroblasts ($n = 18$) (Fig. 6).

DISCUSSION

Dupuytren's disease is a proliferative fibrotic disorder and while its etiology remains unknown, we theorize that it is pathobiologically related to other progressive fibrosing human physiologic disorders such as keloid and proliferative scar formation. All recent evidence suggests that $TGF_{\beta 1}$ and $TGF_{\beta 2}$ are the key cytokines in scarring and fibrotic conditions [8, 9, 27–34]. It has been demonstrated that $TGF_{\beta 2}$ plays an important role in the fibrogenic nature of Dupuytren's disease [2, 12]. Fibroblasts are abundant within the affected palmar fascia and TGF_{β} has been demonstrated to be a stimulus for both collagen and noncollagen production by these cells [7, 14, 35].

Tamoxifen, a synthetic nonsteroidal anti-estrogen, has been demonstrated to have multiple effects includ-

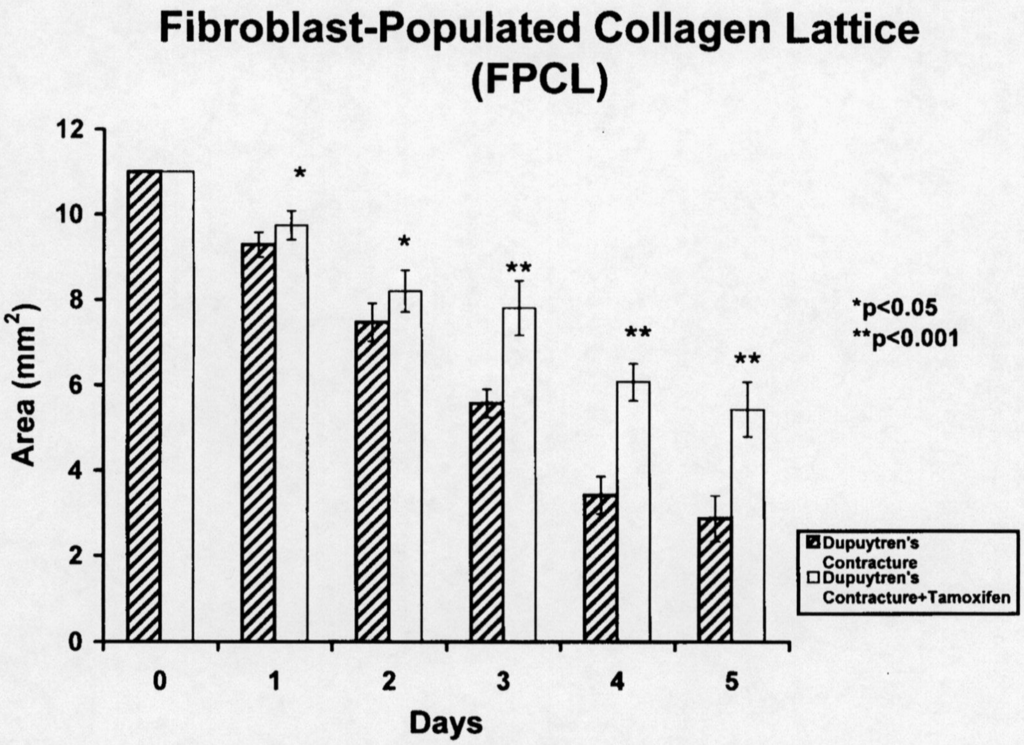

FIG. 2. Comparison of fibroblasts derived from Dupuytren's affected fascia treated with tamoxifen. Fibroblasts treated with tamoxifen had significantly less contraction of a collagen lattice compared to untreated fibroblasts.

ing altered RNA transcription, decreased cellular proliferation, delay or arrest of the cells in the G1 phase of the cell cycle, and interference with multiple growth factors such as TGF_β and IGF [24, 26]. It is the interference with TGF_β that has created interest in the area of manipulating proliferative scarring and the treatment of fibroproliferative disorders. TGF_β is known to contribute to the excessive production of collagen, which in turn leads to fibrosis that is characteristic of fibroproliferative disorders. Inhibition of TGF_β can decrease collagen production [24]. *In vitro* studies have demonstrated that tamoxifen inhibits the proliferation of keloid fibroblasts and their rate of collagen synthesis and decreases their ability to contract a collagen lattice [24, 26]. It has also been reported that benign tumors such as desmoids and retroperitoneal fibrosis have been treated successfully with tamoxifen [17, 26]. Mancoll *et al.* have demonstrated a decreased production of TGF_β in keloid fibroblasts that were treated with tamoxifen [36].

We have been able to demonstrate decreased function of fibroblasts derived from Dupuytren's affected fascia in FPCL. We have also shown a downregulation of $TGF_{\beta2}$ in these same fibroblasts. In carpal tunnel cases (normal), the fascia shows less active fibroblasts in FPCL when treated with tamoxifen but not significantly lower levels of $TGF_{\beta2}$. Although FPCL contraction in the carpal tunnel derived fibroblasts is slightly decreased by tamoxifen, it does not appear to be a result of $TGF_{\beta2}$ downregulation. It is possible that there may be a small direct effect on the fibroblasts such as altered RNA transcription, decreased cellular proliferation, and/or delay or arrest of the cells in the G1 phase of the cell cycle. However, the 8 μmol/ml concentration of tamoxifen used has been shown not to significantly inhibit fibroblast proliferation in collagen lattices [26]. Because the normal fascia control did not respond to tamoxifen with downregulation of $TGF_{\beta2}$, it can then be inferred that the fibrotic nature of Dupuytren's disease is related to activated fibroblasts and secretion of fibroblastic cytokines like $TGF_{\beta1}$ and $TGF_{\beta2}$.

If stimulation of fibrosis by $TGF_{\beta2}$ plays a role in the pathogenesis of Dupuytren's disease, recent data support other forms of treatment as well as tamoxifen. Neutralizing antibodies to $TGF_{\beta1}$ and $TGF_{\beta2}$ have been demonstrated to reduce dermal scarring in rat dermal wounds [37, 38]. $TGF_{\beta2}$ antibody has been demonstrated to decrease contraction of keloid or burn hypertrophic scar fibroblast-populated collagen lattices [39]. These observations have been extended to *in vivo* demonstrations that explanted human proliferative scar collagen production can be downregulated by $TGF_{\beta2}$ antibody [40]. There are other ways to attack overproduction of $TGF_{\beta1}$ and $TGF_{\beta2}$. McCallion and Ferguson have used exogenous mannose 6-phosphate to reduce scarring in rodent, porcine, and human wounds [41]. Mannose 6-phosphate prevents activation of $TGF_{\beta1}$ by

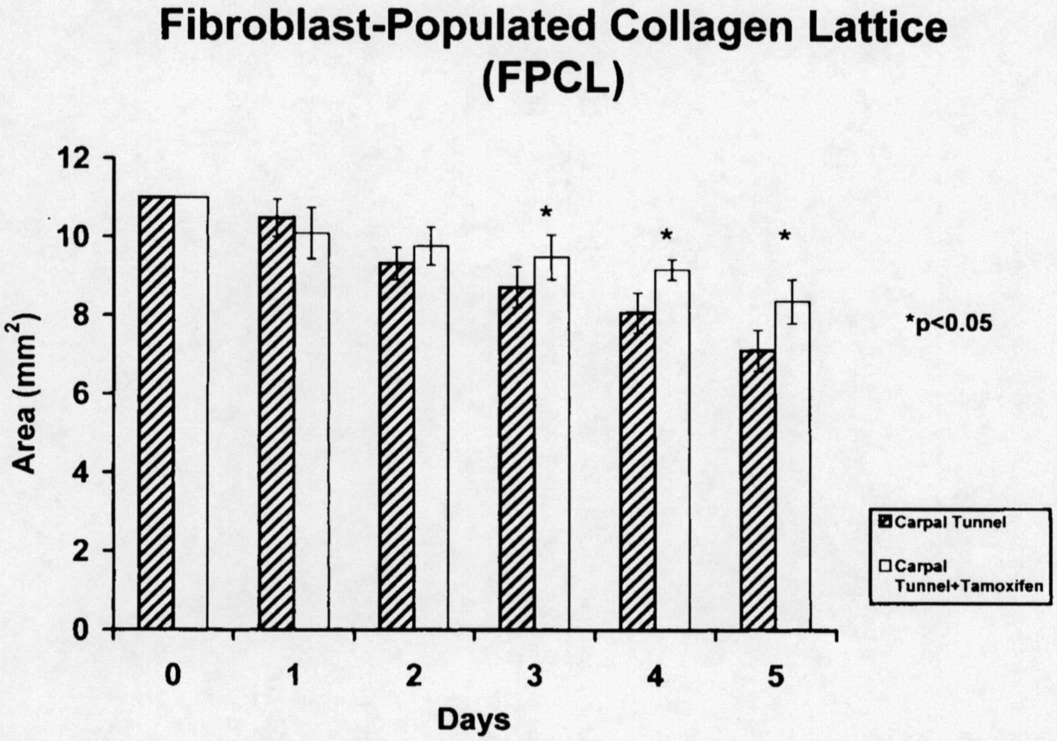

FIG. 3. Carpal tunnel fibroblasts (normal) treated with tamoxifen had significantly less ability to contract a collagen lattice than untreated fibroblasts.

competitive inhibition of binding of the critical latency associated peptide [27]. Decorin is a leucine-rich proteoglycan that is important in collagen fiber formation [42]. Fibroblasts from burn hypertrophic scars produce less decorin than normal dermal fibroblasts [43]. Decorin binds and neutralizes all three TGF_{β} isoforms and the protein has also been used in the central nervous system to decrease fibrotic scarring known to be secondary to $TGF_{\beta 1}$ [44, 45]. Finally, Tredget *et al.* have been able to decrease the volume of hypertrophic scars with the use of systemic interferon α-2b [46]. The action appears to be secondary to the ability of interferon α-2b to normalize elevated levels of $TGF_{\beta 1}$ and $TGF_{\beta 2}$.

Another area of interest that has not been extensively explored is the topical application of tamoxifen. It is known that oral doses of tamoxifen similar to those used in breast cancer have been used successfully for the treatment of desmoid tumors [47, 48]. There has been a comparison of the distribution of tamoxifen metabolites in the plasma and in normal

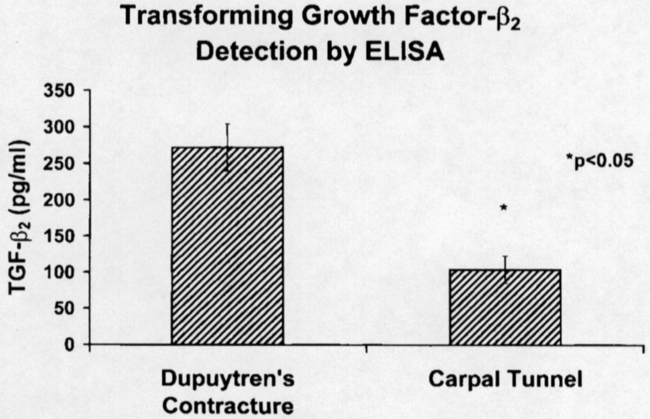

FIG. 4. $TGF_{\beta 2}$ detection ELISA of fibroblasts derived from Dupuytren's affected palmar fascia compared to carpal tunnel derived fibroblasts (normal). There was a significantly greater amount of $TGF_{\beta 2}$ detected in the Dupuytren's contracture group.

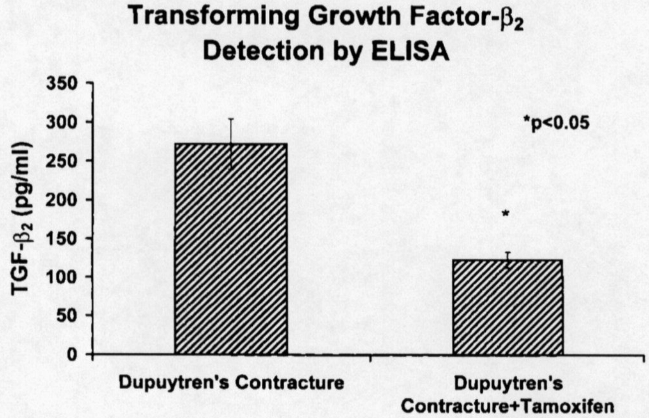

FIG. 5. $TGF_{\beta 2}$ detection ELISA comparing fibroblasts derived from Dupuytren's affect palmar fascia, untreated and treated with tamoxifen. Tamoxifen significantly decreased the amount of $TGF_{\beta 2}$ detected.

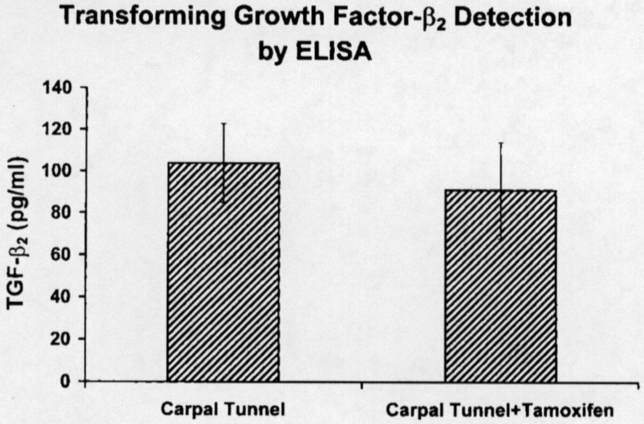

FIG. 6. $TGF_{\beta 2}$ detection ELISA comparing fibroblasts derived from carpal tunnel, untreated and treated with tamoxifen. There was no downregulation of $TGF_{\beta 2}$ detected with tamoxifen treatment.

and tumor breast tissue following oral versus topical application [49]. Comparing topical and oral doses has been difficult and it is believed that various factors influence the rate of hydroxylation in the liver and, subsequently, the proportion of tamoxifen metabolites [26]. It may be possible, with further clinical investigation, to use the topical application of tamoxifen for the treatment of fibroproliferative disorders such as Dupuytren's disease.

Tamoxifen, by neutralizing or downregulating $TGF_{\beta 2}$, may prove to be a method to manipulate and control Dupuytren's contracture in the clinical setting. Eventually, through further clinical investigation, it may be possible to halt or even reverse this progressive and debilitating disease.

REFERENCES

1. Dupuytren, B. Permanent retraction of fingers produced by an affection of the palmar fascia. *Lancet* **2:** 222, 1834.
2. Kloen, P., Jennings, C. L., Gebhardt, M. C., Springfield, D. S., and Mankin, H. J. Transforming growth factor-beta: Possible roles in Dupuytren's contracture. *J. Hand Surg. [Am.]* **20:** 101, 1995.
3. Badalamente, M. A., Hurst, L. C., Grandia, S. K., and Sampson S. P. Platelet-derived growth factor in Dupuytren's disease. *J. Hand Surg. [Am.]* **17:** 317, 1992.
4. Gabbiani, G., and Majno, G. Dupuytren's contracture: Fibroblast contraction? An ultrastructural study. *Am. J. Pathol.* **66:** 131, 1972.
5. Vande Berg, J. S., Gelberman, R. H., Rudolph, R., Johnson, D., and Sicurello, P. Dupuytren's disease: Comparative growth dynamics and morphology between cultured myofibroblasts (nodule) and fibroblasts (cord). *J. Orthop. Res.* **2:** 247, 1984.
6. Rudolph, R., and Vande Berg, J. The myofibroblast in Dupuytren's contracture. *Hand Clin.* **7:** 683, 1991.
7. Berndt, A., Kosmehl, H., Mandel, U., Gabler, U., Luo, X., Celeda, D., Zardi, L., and Katenkamp, D. TGFβ and bFGF synthesis and localization in Dupuytren's disease (nodular palmar fibromatosis) relative to cellular activity, myofibroblast phenotype and oncofetal variants of fibronectin. *Histochem. J.* **27:** 1014, 1995.
8. Border, W. A., and Nobel, N. A., Transforming growth factor beta in tissue fibrosis. *N. Engl. J. Med.* **331:** 1286, 1994.
9. Border, W. A., and Rouslahti, E. Transforming growth factor-beta in disease: The dark side of tissue repair. *J. Clin. Invest.* **90:** 1, 1992.
10. Smith, P. D., Siegler, K., Wang, X., and Robson, M. C. Transforming growth factor β_2 increases DNA synthesis and collagen production in keloid fibroblasts. *Surg. Forum* **49:** 617, 1998.
11. Gonzalez, A. M., Buscaglia, M., Fox, R., Isacchi, A., Sarmientos, P., Farris, J., Ong, M., Martineau, D., Lappi, D. A., and Baird, A. Basic fibroblast growth factor in Dupuytren's contracture. *Am. J. Pathol.* **141:** 661, 1992.
12. Badalamente, M. A., Sampson, S. P., Hurst, L. C., Dowd, A., and Miyasaka, K. The role of transforming growth factor beta in Dupuytren's disease. *J. Hand Surg. [Am.]* **21:** 210, 1996.
13. Magro, G., Lanteri, E., Micali, G., Paravizzini, G., Travali, S., and Lanzafame, S. Myofibroblast of palmar fibromatosis co-express transforming growth factor-alpha and epidermal growth factor receptor. *J. Pathol.* **181:** 213, 1997.
14. Alioto, R. J., Rosier, R. N., Burton, R. I., and Puzas, J. E. Comparative effects of growth factors on fibroblasts of Dupuytren's tissue and normal palmar fascia. *J. Hand Surg. [Am.]* **19:** 442, 1994.
15. Kuhn, M. A., Payne, W. G., Kierney, P. C., Pu, L. L. Q., Smith, P. D., Siegler, K., Ko, F., Wang, X., and Robson, M. C. Cytokine manipulation of explanted Dupuytren's affected human palmar fascia. *Int. J. Surg. Inv.* **2:** 443, 2001.
16. Chau, D., Mancoll, J. S., Lee, S., Zhao, J., Phillips, L. G., Gittes, G. K., and Longaker, M. T. Tamoxifen downregulates TGF-β production in keloid fibroblasts. *Ann. Plast. Surg.* **40:** 490, 1998.
17. Owens, L. V., Cance, W. G., and Huth, J. F. Retroperitoneal fibrosis treated with tamoxifen. *Am. Surg.* **61:** 842, 1995.
18. Clark, C. P., Vanderpool, D., and Preskitt, J. T. The response of retroperitoneal fibrosis to tamoxifen. *Surgery* **109:** 502, 1991.
19. Timmons, M. J. Fibromatosis, desmoids, fibroblasts, and tamoxifen. *Br. J. Plast. Surg.* **47:** 378, 1994.
20. Jordan V. C. Growth factor regulation by tamoxifen is demonstrated in patients with breast cancer. *Cancer* **72:** 1, 1993.
21. Murphy, L. C. Antiestrogen action and growth factor regulation. *Breast Cancer Res. Treat.* **31:** 61, 1994.
22. Dickens, T. A., and Colletta, A. A. The pharmacological manipulation of members of the transforming growth factor beta family in the chemoprevention of breast cancer. *Bioessays* **15:** 71, 1993.
23. Pollock, M., Costantino, J., Polychronakos, C., Blauer, S. A., Guyda, H., Redmond, C., Fisher, B., and Margolese, R. Effect of tamoxifen on serum insulin-like growth factor 1 levels in stage 1 breast cancer patients. *J. Natl. Cancer Inst.* **82:** 1693, 1990.
24. Mancoll, J. S., Zhao, J., McCauley, R. L., and Phillips, L. G. The inhibitory effect of tamoxifen on keloid fibroblasts. *Surg. Forum* **47:** 718, 1996.
25. Rayan, G. M., and Tomasek, J. J. Generation of contractile force by cultured Dupuytren's disease and normal palmar fibroblasts. *Tissue Cell* **26:** 747, 1994.
26. Hu, D., Hughes, M. A., and Cherry, G. W. Topical tamoxifen—A potential therapeutic regime in treating excessive dermal scarring? *Br. J. Plast. Surg.* **51:** 462, 1998.
27. O'Kane, S., and Ferguson, M. W. J. Transforming growth factor βs and wound healing. *Int. J. Biochem. Cell Biol.* **29:** 63, 1997.
28. Younai, S., Nichter, L. S., Wellisz, T., Reinisch, J., Nimni, M. E., and Tuan, T. L. Modulation of collagen synthesis by transform-

ing growth factor-beta in keloid and hypertrophic scar fibroblasts. *Ann. Plast. Surg.* **33:** 148, 1994.

29. Younai, S., Venters, G., Vu, S., Nichter, L., Nimni, M., and Tuan, T. L. Role of growth factors in scar contraction: An in vitro analysis. *Ann. Plast. Surg.* **36:** 495, 1996.

30. Lee, T. Y., Chin, G. S., Kim, W. J. H., Chau, D., Gittes, G. K., and Longaker, M. T. Expression of transforming growth factor beta 1, 2, and 3 proteins in keloids. *Ann Plast Surg.* **43:** 179, 1999.

31. Shah, M., Foreman, D. M., and Ferguson, M. W. Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. *J. Cell Sci.* **108:** 985, 1995.

32. Cox, D. A. Transforming growth factor beta-3. *Cell Biol. Int.* **19:** 357, 1995.

33. Kuhn, A., Singh, S., Smith, P. D., Ko, F., Falcone, R., Lyle, W. G., Maggi, S. P., Wells, K. E., and Robson, M. C. Periprosthetic breast capsules contain the fibrogenic cytokines TGF-$\beta 1$ and TGF-$\beta 2$, suggesting possible new treatment approaches. *Ann. Plast. Surg.* **44:** 387, 2000.

34. Pu, L. L. Q., Smith, P. D., Payne, W. G., Kuhn, M. A., Wang, X., Ko, F., and Robson, M. C. Overexpression of transforming growth factor beta-2 and its receptor in rhinophyma: An alternative mechanism of pathobiology. *Ann. Plast Surg.* **45:** 515, 2000.

35. Igarashi, A, Nashiro, K., Kikuchi, K., Sato, S., Ihn, H., Fujimoto, M., Grotendorst, G. R., and Takehara, K. Connective tissue growth factor gene expression in tissue sections from localized scleroderma, keloid, and other fibrotic skin disorders. *J. Invest. Dermatol.* **106:** 729, 1996.

36. Mancoll, J. S., Chau, D., Munger, J., Harpel, J., Zhao, J., Gittes, G. K., Longaker, M. T., and Phillips, L. G. Tamoxifen downregulates TGF-β production by keloid fibroblasts. *Surg. Forum* **48:** 704, 1997.

37. Shah, M., Foreman, D. M., and Ferguson, M. W. Neutralising antibody to TFG-$\beta_{1,2}$ reduces cutaneous scarring in adult rodents. *J. Cell Sci.* **107:** 1137, 1994.

38. Shah, M., Foreman, D. M., and Ferguson, M. W. J. Control of scarring in adult wounds by neutralising antibody to transforming growth factor β. *Lancet* **339:** 213, 1992.

39. Smith, P., Mosiello, G., Deluca, L., Ko, F., Maggi, S., and Robson, M. C. TGF-β_2 activates proliferative scar fibroblasts. *J. Surg. Res.* **82:** 319, 1999.

40. Wang, X., Smith, P., Pu, L. L. Q., Kim, Y. J., Ko, F., and Robson, M. C. Exogenous TGF-β_2 modulates collagen I and collagen III synthesis in proliferative scar xenografts in nude rats. *J. Surg. Res.* **87:** 194, 1999.

41. McCallion, R. L., and Ferguson, M. W. J. Fetal wound healing and the development of antiscarring therapies for adult wound healing. In R. A. F Clark (Ed.), *The Molecular and Cellular Biology of Wound Repair.* New York: Plenum Press, 1996. Pp. 561–600.

42. Danielson, K. G., Baribault, H., Holmes, D. F., Graham, H., Kadler, K. E., and Iozzo, R. V. Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. *J Cell Biol.* **136:** 729, 1997.

43. Scott, P. G., Dodd, C. M., Ghahary, A., Shen, Y. J., and Tredget, E. E. Fibroblasts from post-burn hypertrophic scar tissue synthesize less decorin than normal dermal fibroblasts. *Clin. Sci.* **94:** 541, 1998.

44. Hildebrand, A., Romaris, M., Rasmussen, L. M., Heinegard, D., Twardzik, D. R., Border, W. A., and Ruoslahti, E. Interaction of the small interstitial proteoglycans biglycan, decorin, and fibromodulin with transforming growth factor beta. *Biochem J.* **302:** 527, 1994.

45. Logan, A., Berry, M., Gonzalez, M., Frautschy, S. A., Sporn, M. B., and Baird, A. Effects of transforming growth factor-$\beta 1$ on scar production in the injured central nervous system of the rat. *Eur. J. Neurosci.* **6:** 355, 1994.

46. Tredget, E. E., Shankowsky, H. A., Pannu, R., Nedelec, B., Iwashina, T., Ghahary, A., Taerum, T. V., and Scott, P. G. Transforming growth factor-β in thermally injured patients with hypertrophic scars: Effects of interferon alpha-2b. *Plast. Reconstr. Surg.* **102:** 1317, 1998.

47. Kinzbrunner, B., Ritter, S., Domingo, J., and Rosenthal, C. J. Remission of rapidly growing desmoid tumors after tamoxifen therapy. *Cancer* **52:** 2201, 1983.

48. Brooks, M. D., Ebbs, S. R., Colletta, A. A., and Baum, M. Desmoid tumours treated with triphenylethylenes. *Eur. J. Cancer* **28A:** 1014, 1992.

49. Pujol, H., Girault, J., Rouanet, P., Fournier, S., Grenier, J., Simony, J., Fourtillan, J. B., and Pujol, J. L. Phase 1 study of percutaneous 4-hydroxytamoxifen with analyses of 4-hydroxytamoxifen concentrations in breast cancer and normal breast tissue. *Cancer Chemother. Pharmacol.* **36:** 493, 1995.