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ORIGINAL ARTICLE
Research

Tamoxifen Upregulates Collagenase Gene Expression in Human Dermal Fibroblasts

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Background: Tamoxifen is a known inhibitor of fibroblast transforming growth factor beta biosynthesis and wound scar formation. Tamoxifen is also known to be an estrogen antagonist and protein kinase C (PKC) inhibitor. Cells treated with tamoxifen and other PKC/calmodulin inhibitors depolymerize their membrane focal adhesion complexes and cytoskeletal protein structures. These effects result in substrate detachment, cell shape rounding, and upregulation of collagenase synthesis and extracellular matrix degradation. The purpose of our study was to test the hypothesis that tamoxifen treatment of human foreskin fibroblasts results in alteration of cytoskeletal protein organization, cell detachment and rounding, and increased collagenase synthesis similar to known PKC/calmodulin inhibitors such as H-7.

Methods: We characterized the effects of PKC/calmodulin inhibitors tamoxifen and H-7 on human dermal fibroblast morphology, cytoskeletal protein organization, and collagenase gene expression in monolayer culture and within collagen gels.

Results: We found that fibroblasts responded to tamoxifen by initiation of actin filament depolymerization followed by alteration from spindle to spheroidal shapes. This change in cell shape led to increased collagenase synthesis in cells treated with either tamoxifen or H-7 compared with controls. There was also a 23% increase of hydroxyproline release from tamoxifen-treated fibroblast-populated collagen matrices.

Conclusions: Tamoxifen may reduce scarring by inhibiting fibroblast PKC/calmodulin activity, which down-regulates pro-fibrotic transforming growth factor beta signaling and upregulates collagenase production. These effects mimic those of the known PKC/calmodulin inhibitor H-7. Overall, these findings suggest that tamoxifen and its analogues are promising agents for clinical investigation as small molecule regulators of fibrosis and scarring disorders. *(Plast Reconstr Surg Glob Open 2024; 12:e5609; doi: 10.1097/GOX.0000000000005609; Published online 12 February 2024.)*

INTRODUCTION

Tamoxifen is a 371.5 Da synthetic derivative of triphenylethylene. It was specifically synthesized to competitively bind to estrogen receptors and function clinically as a nonsteroidal estrogen antagonist agent. Tamoxifen is commercialized for therapeutic intervention in certain breast cancer patient treatments. Tamoxifen has also been reported as a potential interventional therapy for various excess tissue fibrosis disorders, including desmoid tumors, keloids, and hypertrophic scars.[1–5] The mechanism of this scar inhibition has been linked to inhibition of transforming growth factor beta (TGF-β) production.[5–8] TGF-β in fibroblasts enhances collagen production and integrin expression, which contribute to abnormal extracellular matrix (ECM) deposition in scars.[5,7,9]

It has been well established that tamoxifen inhibits both protein kinase C (PKC) and calcium/calmodulin-dependent protein kinase (CaMK) activity.[10,11] PKC and calmodulin are part of the cellular signaling pathway that responds to dynamic mechanical loading through metalloproteinases (MMPs) such as collagenase.[12] MMPs play an important role in degradation of ECM proteins. Tamoxifen inhibits PKC by two different mechanisms: blocking PKC allosteric cofactors calcium and phosphatidylserine (PS) and inhibiting the catalytic domain of

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Received for publication November 10, 2023; accepted December 15, 2023.

DOI: 10.1097/GOX.0000000000005609

Disclosure statements are at the end of this article, following the correspondence information.

PKC by binding to the ATP binding region.[10] Tamoxifen inhibits CaMK activity through competitively binding to the activated CaMK complex.[10,13] Inhibition of PKC and CaMK activity has been shown to reduce TGF-β synthesis,[14–17] thus reducing amount of collagen production, and induce programmed cell death.[15,18] Inhibition of CaMK activity has also been shown to increase interleukin 1 (IL-1) induced collagenase production in fibroblasts.[19]

We postulated that tamoxifen's effect on fibrosis disorders and TGF-β is mediated by PKC and CaMK inhibition. From tamoxifen's inhibition of CaMK and PKC, tamoxifen alters cytoplasmic protein phosphorylation to reduce the cell focal adhesion complex and change cytoskeletal protein structure.[12] The loss of cell adhesion and changes in cell shape results in upregulation of collagenase biosynthesis and ECM degradation through pathways such as TGF-β production.[20,21] Previous investigations in our laboratory have demonstrated that human scar fibroblasts treated with PKC inhibitors and calcium channel antagonists such as nifedipine and high-dose verapamil induce changes in cell shape due to actin filament depolymerization.[22,23] PKC inhibitors and calcium channel antagonists also increased collagenase synthesis.[22–24] We postulated that tamoxifen may act similarly to these PKC inhibitors.

The purpose of this study was to test the hypothesis that tamoxifen treatment of human foreskin fibroblasts results in alteration of cytoskeletal protein organization, cell detachment and rounding, and increased biosynthesis of collagenase similar to known PKC and CaMK inhibitors.

Takeaways

Question: Does tamoxifen treatment of human foreskin fibroblasts result in alteration of cytoskeletal protein organization, cell morphology, and collagenase synthesis similar to known PKC/calmodulin inhibitors?

Findings: We characterized the effects of tamoxifen and known PKC/calmodulin inhibitor H-7 on human fibroblast cytoskeletal protein organization, fibroblast morphology, and collagenase gene expression in monolayer culture and within collagen gels. Our findings showed that fibroblasts responded to tamoxifen by initiation of actin filament depolymerization, cell rounding, and increase in collagenase synthesis, similar to H-7.

Meaning: Tamoxifen may reduce scarring by inhibiting fibroblast PKC/calmodulin activity. These effects mimic those of the known PKC/calmodulin inhibitor H-7.

MATERIALS AND METHODS

Cell Culture

Human foreskin fibroblasts (AG07095) were purchased from Coriell Institute for Medical Research (Camden, N.J.). Passage 5 to 9 cells were grown in monolayer either on plastic culture dishes (Falcon, Lincoln Park, N.J.) or on sterile glass coverslips (Fisher, Itasca, Ill.). They were cultured in the Dulbecco modified Eagle medium (DMEM, Gibco, Grand Island, N.Y.) with 10% fetal bovine serum (FBS, Hyclone, Logan, Utah). Cell viability was high.

Quantification of the Percentage of Cells with Spheroidal Shape

After cells reached about 50%–80% confluence, they were treated with tamoxifen (Sigma, St. Louis, Mo.) in DMEM. PKC inhibitor H-7 (Seikagaku America, Ijamsville, Md.)-treated cells served as positive controls, and cells incubated with only DMEM served as negative controls. To determine whether PKC inhibitor-induced cell rounding is dose-dependent, cells were treated with 1 or 10 μM tamoxifen or 10, 20, 50, and 100 μM H-7 for 90 minutes. Cells were then fixed with 3.75% formaldehyde and examined under an inverted microscope with a 10× or 20× objective lens. To count cell number accurately, we chose a field of view with 30–150 cells under the microscope.

Dual-labeling Technique for the Staining of Actin Filaments and Microtubules

To visualize actin filaments and microtubules after treatment with tamoxifen or H-7, cells were first fixed with 3.75% formaldehyde for 10 minutes, permeabilized with 0.1% Triton X-100 for another 10 minutes, and then incubated with mouse monoclonal anti-α tubulin (Amersham Corp., Arlington Heights, Ill.) at 37°C for 45 minutes. The mouse monoclonal antibody was diluted 500 times with phosphate-buffered saline (PBS) containing 1% FBS. After the mouse anti-α tubulin was discarded, cells were stained with a secondary antibody, Texas red–labeled antimouse immunoglobulin from sheep (Amersham Corp., Arlington Heights, Ill.), for 1 hour. Cells were stained for actin filaments with FITC (fluorescein-5-isothiocyanate)-labeled phalloidin (Molecular Probes/Sigma, St. Louis, Mo.) simultaneously at a concentration of 1 mg per mL at 37°C. Cells were observed under a confocal microscope using a 63× objective lens.

Single- and Dual-labeling for Collagenase

Human fibroblasts were incubated in DMEM containing 1 or 10 μM tamoxifen or 10, 20, 50, 100 μM H-7 at 37°C for 90 minutes. Then, cells recovered from the treatments by changing culture media to fresh DMEM without tamoxifen or H-7. Three hours before the end of the experiment, 5 μM maninis (final concentration, Sigma, St. Louis, Mo.) was added to the culture media to stop the secretion of collagenase. Cells were fixed in 3.75% formaldehyde, and cell membranes were permeabilized with 0.1% Triton X-100 for 10 minutes on a rotary shaker. Triton X-100 was later rinsed off with three PBS washes. The cells were then incubated in 1 ml of sheep antihuman collagenase antiserum at 37°C for 45 minutes. The primary antibody was removed with three PBS washes. The cells were stained with a secondary antibody, FITC-labeled rabbit $F(ab')_2$ antisheep IgG (Southern Biotechnology Associates Inc., Birmingham, Ala.) for another 45 minutes at 37°C. During the secondary antibody staining against collagenase, the cells were co-incubated with 2 units/mL rhodamine phalloidin

(Molecular Probes, Eugene, Oreg.) for actin filaments. After staining, cells were examined with a confocal microscope.

Fluorescence Imaging by Confocal Laser Scanning Microscopy

Confocal images of cells were obtained using an Odyssey XL confocal unit (Noran Instruments, Middleton, Wisc.) connected to a Zeiss AxioVert 135 HDTV microscope. The FITC dye was excited at a wavelength of 488 nm by an Argon/Krypton laser and the emitted light was filtered through a 515 nm barrier filter. For Texas red, the excitation wavelength was 568 nm, and the emission wavelength was 590 nm. Cell images were averaged over 256 frames to improve signal to noise ratios and the brightness and contrast were altered as needed to improve the features of the images. To reconstruct a three-dimensional image, confocal images of the cell were taken at every 0.27 μm (z-axis) for a total thickness of 30 μm.

Quantification of the Percentage of Cells Expressing Increased Collagenase Synthesis

After human dermal fibroblasts were treated with 1 or 10 μM tamoxifen, or 10, 20, 50, or 100 μM H-7 for 90 minutes, cells recovered from the treatments for 24 hours. Then the treated cells and control cells were stained for collagenase using the method described above. Cells were counted under a confocal microscope using a 20× objective lens with 20–50 cells per field view.

The Effect of Tamoxifen on the Dry Weight and the Morphology of FPCM

Free-floating fibroblast-populated collagen matrices (FPCMs) were used as a model system to study whether tamoxifen promotes extracellular matrix degradation by increasing collagenase synthesis for cells embedded in a tissue equivalent. The dry weight of an FPCM was used as an indirect measurement of collagenase activity. Because the major solid component of an FPCM is collagen, a significant increase in collagenase production will decrease the dry weight of an FPCM. The morphology of FPCMs with or without tamoxifen treatment was also observed and recorded photographically. FPCMs were prepared by mixing human dermal fibroblasts, AG 07095, with type I collagen and DMEM containing 10% FBS.[25] Type I collagen, extracted from rat tail tendons, was purified by NaCl precipitation and centrifugation.[26] The cell–collagen mixture was poured into an 80×40 mm Pyrex dish, where it solidified within a minute. FPCMs were incubated in DMEM containing 10% FBS and 10 mM fructose at 37°C and 5% CO_2. FPCMs contracted and formed a circular disk of size similar to a quarter 1 week after the FPCMs were fabricated. One-week-old FPCMs of the experimental set were treated with 10 μM tamoxifen for 5 days while the control FPCMs were maintained in DMEM without tamoxifen. FPCMs were photographed before and 5 days after the tamoxifen treatment. At the end of the experiment, FPCMs were lyophilized and weighed using an Ohaus electronic top loading balance. The resolution of this analytical balance is 0.01 mg, and the precision is ±0.02 mg.

Quantification of Collagen Breakdown by Hydroxyproline Assay

A second method used to determine whether tamoxifen stimulates collagenase synthesis involved examining the amount of collagen breakdown products presented in the FPCM culture medium. The collagen breakdown products can be collected by centrifugal filtration and then quantified by a hydroxyproline assay. One-week-old FPCMs were incubated with DMEM or DMEM containing 10 μM tamoxifen for 5 days, and the culture media was collected. Fractions of the culture media were filtered through Millipore Ultra free-MC DEAE filters (Bedford, Mass.). Intact collagen molecules will not be filtered through the DEAE membrane after centrifugation at 5000g for 5–10 minutes but the collagen breakdown products will. The collagen breakdown products in the filtrate were then analyzed for hydroxyproline content spectrophotometrically.[27] The samples were read at 550 nm by a spectrophotometer and the total amount of hydroxyproline of the breakdown collagen presented in the FPCM culture media were calculated. The equation used to determine the total amount of hydroxyproline (micrograms), derived from calibration studies with series dilutions of known hydroxyproline concentrations, is (the absorption at 550 nm − 0.0116)/0.0517×25.

Statistical Analysis

Data are expressed as the mean ± standard error of the mean and was found to be parametric. Comparisons between two means were performed with Student t test using SigmaPlot software (Jandel Scientific, San Rafael, Calif.). Comparisons among three or more means were performed using one-way analysis of variance (ANOVA); where significant differences were detected, t tests with a Bonferroni correction was used for further data exploration. A P value of 0.05 or less was considered to represent a significant difference.

RESULTS

Tamoxifen and H-7-altered Cell Morphology

Human dermal fibroblasts used for these experiments exhibited characteristic fibroblast morphology and mobility in culture. When tamoxifen and PKC inhibitor H-7 were added to the cell media, the fibroblast cell morphology changed from a spindle to spheroidal shape. The effects of tamoxifen and H-7 on cell shape were dose-dependent (Table 1). Higher concentrations of tamoxifen or H-7 caused a higher proportion of cells to change shape. In all cases the mean values for experimental groups were significantly different from the control.

Tamoxifen and H-7-induced Depolymerization of Actin Filaments

Both tamoxifen and PKC inhibitor H-7 induced the depolymerization of actin filaments in human dermal fibroblasts. Figure 1A shows the actin filaments in control fibroblasts cultured in DMEM. Prominent actin stress fibers can be clearly identified (Fig. 1A). After cells were

Table 1. The Percentage of Cells with Spheroidal Shape after Treatment with Tamoxifen or PKC Inhibitor H-7

Treatment	Sample Size	% of Cells with Spheroidal Shape
Control	3106	2.8±1.3
Tamoxifen (1 μM)	2085	13.4±3.6
Tamoxifen (10 μM)	3629	93.3±5.5
PKC inhibitor H-7 (10 μM)	4037	23.7±12.6
PKC inhibitor H-7 (20 μM)	3472	32.5±17.0
PKC inhibitor H-7 (50 μM)	2642	59.4±17.3
PKC inhibitor H-7 (100 μM)	2967	71.2±14.9

treated with either tamoxifen (10 μM) or H-7 (20 μM), actin filaments were completely depolymerized, and some of them reorganized to form small aggregates (Fig. 1B, C).

Using double labeling of actin filaments and microtubules, one can examine both cytoskeletal structures simultaneously. Figure 2A shows the cytoskeletal structures of fibroblasts in the control group (Fig. 2A). Prominent arrays of actin filaments and microtubules can be clearly identified. In serum-supplemented DMEM, their actin filaments and microtubules were distributed throughout the cytoplasm in patterns consistent with those previously described.[28]

After the cells were incubated with tamoxifen or H-7, they became less multipolar, and the actin filaments depolymerized (Fig. 2B, C). Disrupted actin filaments formed small aggregates inside the cell (the green dotted area in Fig. 2B, C). Tamoxifen and H-7 had no effect on microtubules (Fig. 2B, C).

Tamoxifen and H-7-induced Collagenase Synthesis

Increased collagenase synthesis occurred after the PKC-inhibitor-induced cell rounding. Fibroblasts that were allowed to recover following 1-hour treatments with tamoxifen or H-7, by removing the PKC inhibitors from the incubation environment, regained their multipolar shape and exhibited increased collagenase synthesis. Figure 3A shows single-labeled confocal cells in the control group (Fig. 3A). Although the whole cells are stained lightly, no bright spots were observed, indicating little or no collagenase synthesis. In contrast, many bright, intensified spots were seen in tamoxifen- (Fig. 3B) or H-7- (Fig. 3C) treated cells. Figure 4A shows a single-labeled confocal image of a cell in the control group under higher magnification (using 63X objective lens). Once again, there are no particularly bright spots observed, indicating little or no collagenase synthesis (Fig. 4A). When tamoxifen-treated cells exhibiting increased collagenase synthesis were examined under this higher magnification, the labeled locations were observed outside the nucleus (Fig. 4B). Figure 5 shows a composite image of actin filaments and collagenase in the tamoxifen-treated cells (Fig. 5). Cells regained their multipolar shape and re-polymerized their actin filaments after the recovery from tamoxifen treatment.

The number of cells showing increased collagenase synthesis was determined by counting the cells with fluorescent spots in the cytosol. Both tamoxifen and PKC inhibitor H-7 increased the percentage of cells that expressed collagenase synthesis. Tamoxifen at 10 μM and H-7 at 100 μM concentration produced the highest percentages of cells that expressed collagenase synthesis (Table 2). Although the means of the 10 and 20 μM H-7 treated groups were not significantly different ($P = 0.096$), all the means of the PKC inhibitor-treated cells were significantly different from that of controls.

Tamoxifen Decreased Dry Weight and Changed Morphology of FPCMs

The mean dry weight of tamoxifen-treated FPCMs was 5.69±1.33 (N = 27), and the mean dry weight of control FPCMs was 7.96±2.64 (N = 26), a 29% difference. The two means were significantly different ($P < 0.01$).

The tamoxifen-treated FPCMs also exhibited gross morphological differences from the control FPCMs. The control FPCMs were usually intact, smaller, and thicker, visibly more solid, and whitish (Fig. 6A). The tamoxifen-treated FPCMs, in contrast, were looser, larger, thinner, softer, and grayish (Fig. 6B, C). Areas of collagen matrix breakdown could occasionally be identified (Fig. 6B, C).

Quantification of Collagen Degradation by Hydroxyproline Assay

The total amount of hydroxyproline of the collagen breakdown products released from the tamoxifen-treated FPCMs was 154.62±35.45 μg (N = 27). In comparison, the hydroxyproline of collagen breakdown products released from the control FPCMs was 125.78±26.77 μg (N = 26). There was about 23% more hydroxyproline of the collagen

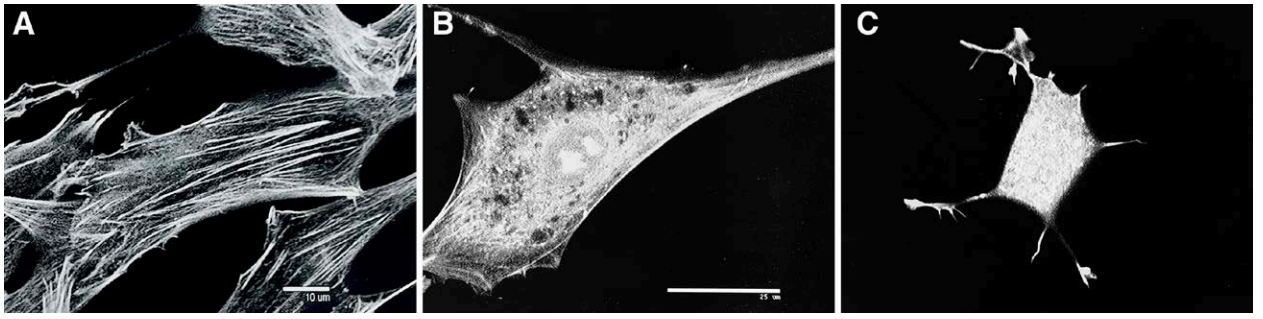

Fig. 1. Single-labeled confocal images of actin filaments in fibroblasts. Organized actin filaments are seen in control fibroblasts (A). In contrast, depolymerized actin filaments are shown in (B) 10 μM tamoxifen-treated cells and (C) 20 μM H-7-treated cells.

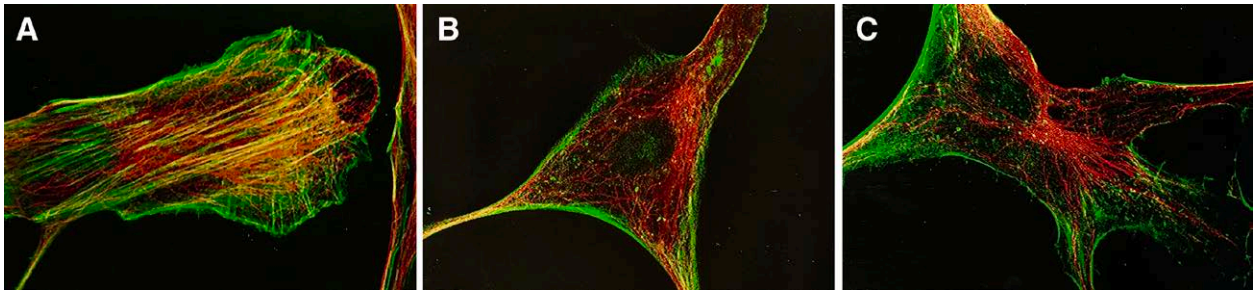

Fig. 2. Double-labeled images of human foreskin fibroblast with 63x magnification. Actin filaments labeled with FITC-labeled phalloidin are in green, and microtubules labeled with anti-α tubulin and Texas red are in red. (A) Control set. (B) Tamoxifen-treated cells. (C) H-7-treated cells. Actin filaments in (B) tamoxifen-treated and (C) H-7-treated fibroblasts were completely depolymerized after incubation for an hour.

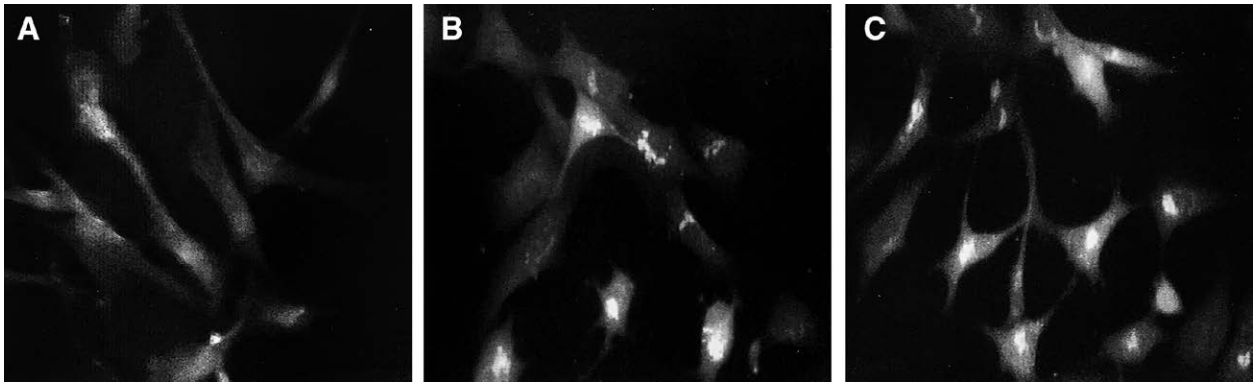

Fig. 3. Single-labeled confocal images of cells that express collagenase synthesis with 20x magnification. (A) Control set. (B) Tamoxifen-treated cells. (C) H-7-treated cells. Bright areas indicating collagenase synthesis can be seen in many (B) tamoxifen-treated and (C) H-7-treated cells.

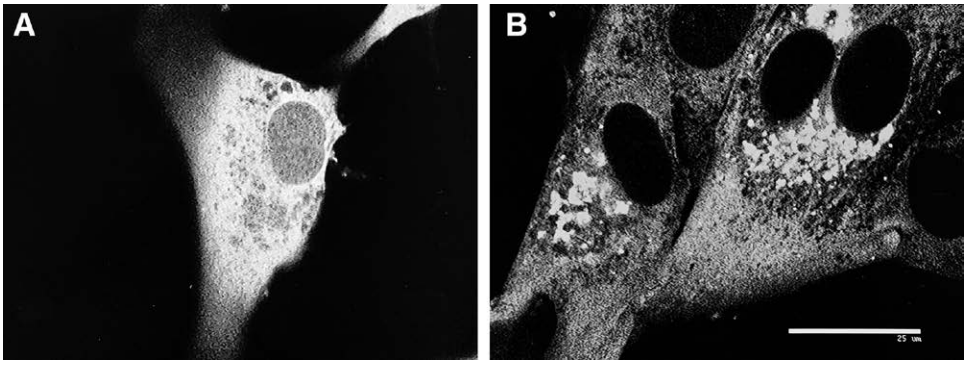

Fig. 4. Single-labeled confocal images of cells that express collagenase synthesis with 63× magnification. No collagenase synthesis is seen in the control cell (A). In contrast, increased collagenase synthesis is seen in the tamoxifen-treated cells (B). Bright, intensified spots inside cells represents areas of collagenase synthesis (B). Black circle in the middle of the cell is the nucleus.

breakdown products in the tamoxifen-treated FPCM culture media than in the control FPCM culture media. The two means were significantly different ($P < 0.01$).

DISCUSSION

We observed that tamoxifen induces human dermal fibroblast rounding and upregulates collagenase production, similar to PKC inhibitor H-7. This observation that tamoxifen mimics the effect of PKC inhibitors has not been previously documented. Both the percentage of cells with spheroidal shape and the percentage of cells expressing collagenase synthesis increased from tamoxifen or H-7 administration in a dose-dependent fashion, suggesting a link between the induction of collagenase synthesis and fibroblast morphology. This linkage

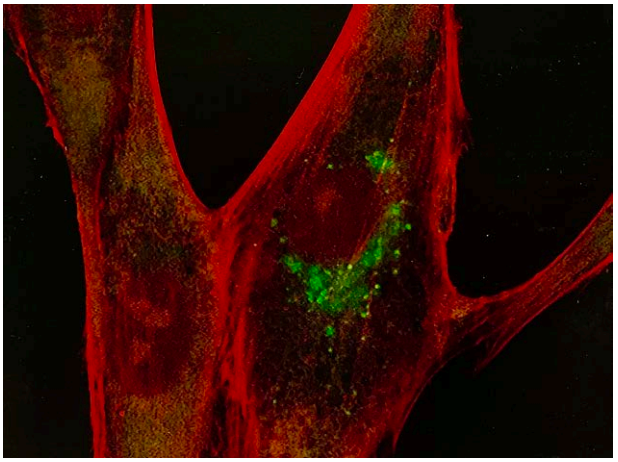

Fig. 5. Composite image of collagenase and re-polymerized actin filaments in the cells that were recovered from tamoxifen treatment with 63× magnification. Red features represent actin filaments, and bright green dots indicate collagenase.

Table 2. The Percentage of Cells with Increased Collagenase Synthesis after Treatment with Tamoxifen or PKC Inhibitor H-7

Treatment	Sample Size	% of Cells with Increased Collagenase Synthesis
Control	417	3.9 ± 2.9
Tamoxifen (1 μM)	514	6.5 ± 3.4*
Tamoxifen (10 μM)	479	61.7 ± 14.2
PKC inhibitor H-7 (10 μM)	434	9.7 ± 6.3†
PKC inhibitor H-7 (20 μM)	446	13.5 ± 6.7
PKC inhibitor H-7 (50 μM)	488	34.1 ± 11.9
PKC inhibitor H-7 (100 μM)	507	57.8 ± 9.7

*The mean is significantly different from the controls ($P = 0.024$).
†The mean is also significantly different from the controls ($P = 0.0017$).

has been well documented in fibroblast cells.[28] Human dermal fibroblasts used in this study respond to tamoxifen and H-7 in a similar fashion as calcium channel blockers and calmodulin inhibitors.[22,23]

The effect of tamoxifen in promoting extracellular matrix degradation was also apparent when examining the integrity of the tamoxifen-treated FPCMs. Collagen matrix breakdown can be seen in the tamoxifen-treated FPCMs (Fig. 6B, C). The diameter of FPCMs after tamoxifen treatment was usually larger than that of the control FPCMs. This is because the fibroblasts residing in the collagen matrix stopped contracting the collagen gel. The contraction of collagen gel is thought to be caused by the mobilization of fibroblasts, which requires reorganization of actin filaments. The complete disruption of actin filaments in the tamoxifen-treated cells would cause the immobilization of the fibroblasts that were embedded in FPCMs and result in the interruption of collagen gel contraction.

The signaling pathways inhibited by tamoxifen mediate fibroblast responses to mechanical stress. It is well established that mechanical tension acting on fibroblasts in promotes scar formation, such as hypertrophic scars and keloids.[21,29] Within this mechanotransduction pathway,

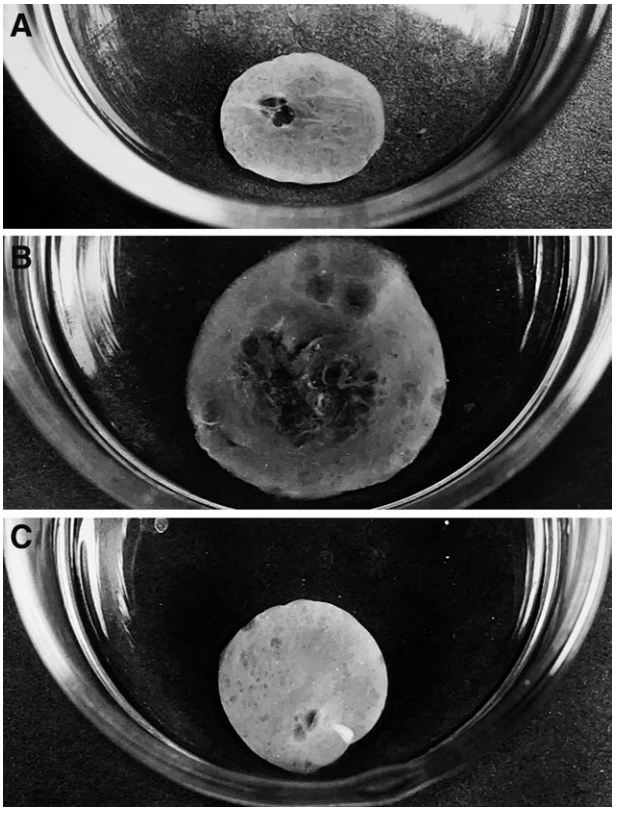

Fig. 6. Morphology of FPCMs after being maintained in DMEM or tamoxifen for 5 days. (A) Control FPCM maintained in DMEM. (B, C) Tamoxifen-treated FPCMs. Tamoxifen-treated FPCMs show collagen matrix breakdown in the middle (B, C).

mechanical stimulation activates mechanosensors such as stretch-activated ion channels and transient receptor potential channels.[12,21,30–32] Activation of these channels leads to an increase in intracellular calcium which can bind to calmodulin to activate Ca/CaM-dependent protein kinases. Mechanical stimulation may also activate G-protein coupled receptors. The activated Gq subtype activates phospholipase C, which cleaves phosphatidylinositol-bisphosphate (PIP2) into inositol-triphosphate (IP3) and diacylglycerol (DAG).[12] These second messengers result in calcium release from the endoplasmic reticulum and PKC activation. Activated Ca/CaM-dependent kinase and PKC alter cytoskeletal structures and regulate gene expression in response to the mechanical loading.[12] Tamoxifen's inhibition of PKC activity inhibits the mechanotransduction pathway. In doing so, tamoxifen prevents cytoplasmic protein phosphorylation involved in cell adhesion during mechanical loading causing the cell shape to round. As our results indicate, this change in cell shape also alters collagenase synthesis. Therefore, the PKC and Ca/CaM-dependent kinase pathways play important roles in responding to human fibroblast cell movement and growth in tissue.

Tamoxifen's efficacy as a promoter of procollagenase synthesis and subsequent extracellular collagen degradation suggest that clinical trials of tamoxifen should be considered to determine tamoxifen (and/or appropriate pharmacological analogies) safety and efficacy to control

fibrosis disorders such as desmoid tumors, keloids, and certain hypertrophic scars. Desmoid tumors are characterized by spindle-shape fibroblasts and abundant collagen bundles; they infiltrate muscle and become densely adherent to major blood vessels and nerves.[33,34] Tamoxifen has been reported to be effective in experimental models of abnormal scarring through topical[5,6] and intralesional[3] application. Our results suggest that tamoxifen exerts its effects through its inhibition of PKC, thus promoting ECM degradation and programmed cell death. Clarifying tamoxifen's effects may elucidate its potential for clinical use.

CONCLUSIONS

Tamoxifen and other PKC inhibitors such as H-7 exert their effects on cell shape, cytoskeletal organization, and collagenase synthesis. These effects promote the degradation of extracellular matrices. Tamoxifen may serve as an important agent for pharmacological control of desmoid tumors, keloids, and hypertrophic scars.

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DISCLOSURES

Dr. Lee is a cofounder of Avocet Polymer Technologies, Inc, Chicago, Illinois. (Avocet has no financial interest in tamoxifen or related technology.) The other authors have no financial interest to declare in relation to the content of this article.

ACKNOWLEDGMENTS

The authors thank Howard Doong, PhD, who initiated this investigation as a part of his doctoral thesis at the University of Chicago; Rosalind Hembry, PhD, for preparing antihuman collagenase antiserum; Prof. Eugene Chang, MD, for the use of confocal microscope, and Profs. Paul Ehrlich, PhD and Robert Walter, PhD, for valuable suggestions. Much of this research was funded by a grant from the American Society for Maxillofacial Surgery (Lee PI).

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