Prostaglandin E_2 Affects Proliferation and Collagen Synthesis by Human Patellar Tendon Fibroblasts

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Objective: To determine the effect of prostaglandin E_2 on proliferation and collagen synthesis by human patellar tendon fibroblasts.

Design and Setting: Controlled laboratory study.

Methods: Human patellar tendon fibroblasts were treated with different concentrations (1, 10, 100 ng/mL) of prostaglandin E_2 in cultures. Fibroblasts without prostaglandin E_2 treatment were used as the control group. The fibroblast proliferation and collagen synthesis were measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay and Sircol collagen assay, respectively.

Main Outcome Measured: Changes in proliferation and collagen production of human patellar tendon fibroblasts.

Results: At 1 ng/mL of prostaglandin E_2, there was no significant effect on fibroblast proliferation compared with the control group. At concentrations of 10 ng/mL and 100 ng/mL prostaglandin E_2, however, fibroblast proliferation significantly decreased, by 7.3% ($P = 0.002$) and 10.8% ($P < 0.0001$), respectively, compared with the control group. At 1 ng/mL of prostaglandin E_2, collagen production of the tendon fibroblasts was unaffected. However, at both 10 ng/mL and 100 ng/mL prostaglandin E_2, collagen production was significantly decreased, by 45.2% ($P < 0.0001$) and 45.7% ($P < 0.0001$), respectively, compared with the control group. The levels of collagen production between these 2 dosages did not differ significantly.

Conclusions: Prostaglandin E_2 affects the proliferation of and collagen production by human patellar tendon fibroblasts in a dosage-dependent manner.

Clinical Relevance: Based on these in vitro findings, we speculate that production of prostaglandin E_2 in tendons might play some role in the acellularity and matrix disorganization seen in exercise-induced tendinopathy.

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Tendinopathy refers to a spectrum of tendon disorders characterized by inflammation and/or degeneration, which may lead to tendon rupture.[1] Excessive, repetitive mechanical loading of the tendon is thought to be one of the major factors involved in the development of this group of disorders.[2] Tendinopathy comprises a large percentage of nontraumatic injuries common in occupational settings and sports.[3] The cellular and molecular mechanisms of tendinopathy, however, are not clear. In an effort to understand the mechanisms better, in vitro studies have been conducted and have shown that cyclic mechanical stretching of human tendon fibroblasts increases the production of prostaglandin E_2 (PGE_2).[4,5] An in vivo study using microdialysis techniques also showed that PGE_2 levels in the peritendinous space were increased after exercise in human subjects.[6] These findings suggest that PGE_2 might be involved in the development of tendinopathy due to repetitive mechanical loading of the tendon.

Prostaglandin E_2 is synthesized from arachidonic acid via a series of intermediate steps.[7] Although it is primarily known as a potent mediator of tissue inflammation,[8] PGE_2 has multiple biologic effects. Elevated concentrations of prostaglandins are found in the joint fluid with inflammatory arthritis.[9] Other studies have shown that PGE_2 regulates proliferation and collagen synthesis in human lung fibroblasts.[10,11] However, it is not known if prostaglandin E_2 has similar effects on human patellar tendon fibroblasts (HPTFs). If PGE_2 affects cell proliferation and collagen production in tendons, then this may explain why the tendinopathic tendon is relatively acellular and has degenerative collagen matrix.[1] Therefore, the purpose of this study was to determine the effects of PGE_2 on the proliferation of and collagen synthesis by human tendon fibroblasts in culture. Based on previous studies in the literature, we hypothesized that exogenous addition of PGE_2 to cultured fibroblasts would inhibit proliferation and collagen production of HPTFs in a dosage-dependent manner. Herein we report the findings of this study.

MATERIALS AND METHODS

Cell Culture

Human patellar tendon fibroblasts or human patellar tenocytes were derived from the tendon samples of young, healthy donors (male, 18 and 20 years old) using explant tissue culture techniques.[5] The samples were obtained from fresh surgical wastes of normal tendon autografts for reconstruction of the anterior cruciate ligament. The protocol for obtaining the tendon samples was approved by the University of Pittsburgh Institutional Review Board (IRB# 0108109). Briefly, tendon samples were minced aseptically, transferred to a 100-mm polystyrene petri dish, and cultured in Dulbecco Modified Eagle Medium supplemented with 10% fetal bovine serum, penicillin (50 U/mL), and streptomycin (50 U/mL). The culture was maintained in a humidified atmosphere of 5% CO_2 at 37°C. To obtain enough fibroblasts for experiments, the cells were subcultured to 4 to 6 passages. The tendon fibroblasts maintained their morphology and doubling time after these passages.

Proliferation Experiments

Fibroblasts were trypsinized from the cultures, counted with a hemacytometer, and plated in 6-well plates to attain about 50% confluence (6×10^4 cells per well). The cells were incubated at 37°C in a humidified atmosphere of 95% CO_2 for 24 hours in growth medium to allow them to become attached and evenly distributed in the wells. After culturing for an additional 24 hours, PGE_2 (Sigma, St. Louis, MO) was added to the wells of the plates. Three different concentrations of PGE_2 (1, 10, and 100 ng/mL) were used for the experimental groups. Fibroblasts without PGE_2 treatment were used as the control group. All the cells in the experimental and control groups were incubated for an additional 48 hours. To determine numbers of viable cells in cultures, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used.[12] Briefly, 200 μL of MTT solution (5 mg/mL) was added to the medium of each well. These were then incubated at 37°C and 5% CO_2 for 3 hours. The supernatant was aspirated, and 3 mL of extraction buffer (15 mL DMF, 14.1 mL H_2O, and 6 g SDS) was added to each well. After overnight incubation at 37°C, the solution was mixed thoroughly. Duplicate samples of 200 μL were divided into a 96-well plate, and the absorbance, or optical density (OD) value, was measured using a microplate reader (Spectra MAX 190; Molecular Devices, CA) at 550 nm. The OD value represented the number of viable cells in each sample. Four sets of separate experiments were performed, with a total of 24 samples for each treatment group.

Collagen Synthesis Experiments

Human patellar tendon fibroblasts were plated in each well of 4 separate 6-well plates, with 10^5 fibroblasts in each well. This high cell density was used to ensure that the cells were confluent, so that cell proliferation was minimized, whereas collagen synthesis was maximized. Also, to promote collagen synthesis, 25 μg/mL ascorbic acid (Sigma) was added to the growth medium in the wells at the time of plating. The cells were then incubated in this medium for 48 hours. After 48 hours, PGE_2, with 3 dosages of 1, 10, and 100 ng/mL, was added to wells of the plates. The control group did not receive PGE_2. The cells were incubated in this medium for an additional 72 hours. Then, the medium in each well was collected to measure total collagen levels in medium samples using Sircol collagen assay (Biocolor Assays, Ireland). This assay is a quantitative dye-binding method designed for the analysis of total collagen. The dye reagent contains Sirius red, which is an anionic dye with a sulphonic acid side-chain group. These groups react with the side-chain groups of the basic amino acids present in collagen. The samples of the medium from the wells were prepared for assay by mixing with Sircol dye reagent for 30 minutes on an orbital shaker. The samples were then centrifuged to collect the collagen-dye complex. The dye bound to the collagen pellet was solubilized with an alkali reagent, and the absorbance of the samples was measured at 540 nm using a microplate reader (Spectra MAX 190). A calibration standard of acid-soluble type I collagen was used to obtain the standard curve. Three sets of separate experiments for collagen synthesis were performed, with a total of 18 samples. In parallel experiments, cell numbers in cultures were determined by MTT assays. They were used to normalize the amounts of collagen measured by Sircol collagen assay. For statistical analysis, one-way ANOVA was used, followed by the Duncan test for multiple comparisons. A P value less than 0.05 was considered statistically significant.

RESULTS

At all three dosages of PGE_2 (1, 10, 100 ng/mL), the morphology of the tendon fibroblasts appeared similar to that of the cells without PGE_2 treatment (Fig. 1).

Cell proliferation at 1 ng/mL PGE_2 was not significantly different from that of the control group ($P = 0.265$). At the concentrations of 10 ng/mL and 100 ng/mL, however, fibroblast proliferation was significantly decreased, by 7.3% ($P = 0.002$) and 10.8% ($P < 0.0001$), respectively, compared with that the cells without PGE_2 treatment. The fibroblast proliferation at 100 ng/mL was not significantly different from that at 10 ng/mL PGE_2 ($P = 0.117$; Fig. 2).

With regard to collagen production, at 1 ng/mL PGE_2, there was no significant difference in collagen levels as compared with the control group ($P = 0.728$). However, at higher PGE_2 concentrations (10 ng/mL and 100 ng/mL), collagen production in medium was significantly decreased, by 45.2% ($P < 0.0001$) and 45.7% ($P < 0.0001$), respectively, compared with the control group. There was no statistical difference in collagen production between PGE_2 concentrations of 10 ng/mL and 100 ng/mL ($P = 0.854$; Fig. 3).

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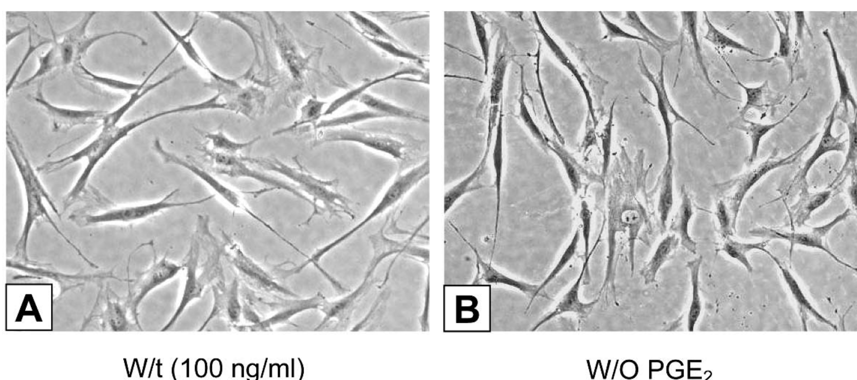

FIGURE 1. Human patellar tendon fibroblasts were cultured in the presence of PGE_2. It is seen that cells treated with the highest dosage of PGE_2 used in this study (A) look similar to those cells without PGE_2 treatment (B). This suggests that the possible toxic effect of PGE_2 on the tendon fibroblasts was minimal.

DISCUSSION

This study shows that exogenous PGE_2 decreased proliferation of HPTFs in vitro. This effect appears to be dose-dependent—that is, increased PGE_2 concentration is associated with decreased cell proliferation. Also, the presence of PGE_2 equal to or greater than 10 ng/mL led to decreased collagen production compared with the control group. The decrease of collagen production was not due to decreased cell proliferation by PGE_2, because the collagen levels in cultures were normalized with respect to cell numbers. Also, the decrease in cell proliferation and collagen synthesis was not due to the possible toxic effects of PGE_2, because cell morphology at the highest PGE_2 dosage (100 ng/mL) used in this study was apparently not changed (Fig. 1).

To the best of our knowledge, there are no studies in the literature about the effects of PGE_2 on human tendon fibroblasts. Therefore, our results cannot be compared directly with other studies. However, there is ample evidence that suggests that prostaglandins affect human fibroblasts derived from other tissues.[13] For example, the addition of PGE_2 leads to a dose-dependent decrease in human lung fibroblast proliferation.[14] The production of prostaglandins by monocytes is thought to be an important regulatory mechanism for the control of lung fibroblast proliferation.[15] Finally, it has been shown that even brief exposure to PGE_2 can decrease fibroblast proliferation by leading to changes in the cell cycle.[16]

Although the molecular mechanisms by which PGE_2 exerts its effects on the tendon fibroblasts in this study are not clear, several types of PGE_2 receptors might be involved.[17–19] Since we did not find difference in fibroblast proliferation and collagen production between 10 and 100 ng/mL PGE_2 concentration, it is possible that the PGE_2 at the concentrations above 10 ng/mL already saturates its receptors, and therefore, no further inhibitory effects can be induced. This possibility remains to be studied.

With regard to the effects of PGE_2 on collagen production, it has been previously shown that prostaglandin E_1 decreases collagen synthesis of human lung fibroblasts.[20] There is also evidence to indicate that cytokine-induced decrease in collagen expression by fibroblasts from other tissues partially involves PGE_2.[21] This effect is not unique to lung fibroblasts,

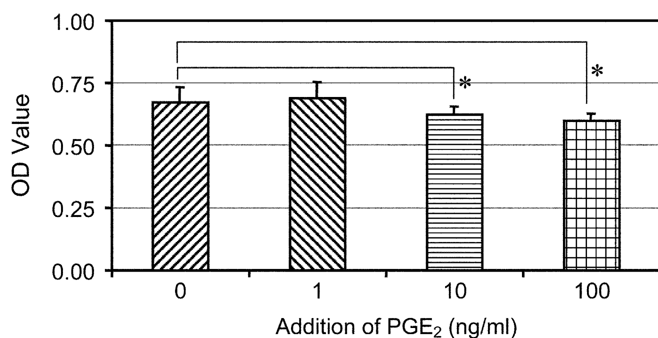

FIGURE 2. Effect of PGE_2 on the proliferation of HPTFs. Higher dosages of PGE_2 (10 and 100 ng/mL) significantly decreased the fibroblast proliferation (*$P < 0.01$). Note that OD values represent the numbers of viable cells in culture.

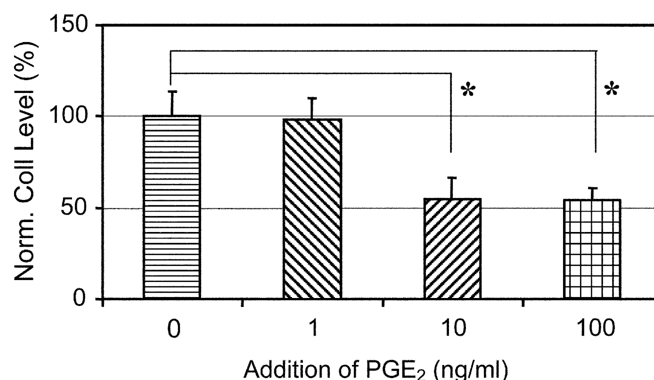

FIGURE 3. Effect of PGE_2 on the collagen production of HPTFs. The addition of PGE_2 at 10 and 100 ng/mL significantly decreased collagen production by the fibroblasts (*$P < 0.0001$). Note that collagen levels were normalized by cell numbers and are represented by percentage changes with respect to those of the fibroblasts without PGE_2 treatment.

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since prostaglandins are also thought to be involved in bradykinin-induced reduction of collagen expression by cardiac fibroblasts.[22] PGE_2 negates the effects of transforming growth factor-β on collagen synthesis in fibroblasts, but interestingly, it does not decrease the amount of collagen produced in response to exogenous insulin, even though the mRNA expression of collagen was decreased.[10] This suggests that there are most likely multiple pathways involved in the synthesis of collagen in response to exogenous chemical signals. However, there is little information in the literature about how human tendon fibroblasts respond to PGE_2 with respect to collagen production. Future studies are warranted to elucidate the molecular mechanisms involved in the PGE_2 effect on collagen production by human tendon fibroblasts.

Fibroblast proliferation and collagen production are two cellular events important to understand the etiology of tendinopathy. Previous studies showed that human tendon fibroblasts produce PGE_2 when subjected to repetitive mechanical stretching.[4,5,23] Also, PGE_2 is produced around tendons of exercising human subjects.[6] Given the fact that tendon fibroblasts are capable of producing PGE_2 under mechanical loading conditions, understanding the effect of PGE_2 on fibroblast proliferation and collagen production in the tendon may shed new light on the developmental mechanisms of tendinopathy. If the PGE_2 produced in the tendon leads to decreased cell proliferation and collagen synthesis, there would not be enough fibroblasts to produce enough collagen for repairing/remodeling matrix in response to repetitive mechanical loading of the tendon. This may result in tendon matrix disorganization and degeneration. Many histologic studies have demonstrated that biopsy specimens from tendinopathic tendons are characterized by acellularity and degenerated collagen matrix that is disorganized and of poor quality.[1,24] Further, as a result of decreased fibroblast proliferation and collagen production, the mechanical properties of a tendon could decrease so that tendon rupture occurs.

It should be noted, however, that PGE_2 is only one of possible factors that mediate the development of tendinopathy. There are many other intrinsic and extrinsic factors that contribute to the etiology of tendinopathy (see review[25]). The multifactor nature of tendinopathy may explain why chronic tendon lesions often show an increase, instead of a decrease as suggested by this study, in cellularity.[26]

There are a few limitations in this study. First, note that tendon fibroblasts within a tendon are organized parallel to collagen fibers, and they are relatively few and far between. In culture, however, the fibroblasts were not organized, and their numbers were relatively high. Therefore, the phenotypic expression of tendon fibroblasts in culture may not be completely representative of that of the cells in vivo. Second, this study examined cell proliferation and collagen synthesis at only one time point (i.e., 72 hours for proliferation and 120 hours for collagen synthesis). Therefore, the kinetics of the PGE_2 effects on the fibroblast proliferation and collagen synthesis are unknown and need to be investigated in future studies. Also, animal models are needed to assess the effect of PGE_2 on tendon in vivo. In conclusion, we have shown that PGE_2 can decrease the proliferation of and collagen production by HPTFs in vitro. These findings may be important in better understanding of the cellular and molecular processes that lead to the development of tendinopathy.

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