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Repeated Exposure of Tendon to Prostaglandin-E$_2$ Leads to Localized Tendon Degeneration

Mustafa H. Khan, MD, Zhaozhu Li, MD, and James H.-C. Wang, PhD

Objective: To determine whether repeated exposure of rabbit patellar tendon to prostaglandin-E$_2$ leads to degenerative changes in the tendon.

Setting: Laboratory animal study.

Main Outcome Measures: Intratendinous changes including cellularity, matrix organization, collagen fibril packing, and diameter.

Methods: Skeletally mature New Zealand White rabbits (n = 10) were transcutaneously injected in the midsubstance of the patellar tendon with prostaglandin-E$_2$ (PGE$_2$; 50 ng or 500 ng). The contralateral tendons were used as 3 different controls (no injection, saline injection, and needlestick only). The injection was repeated once a week for 4 weeks, and the rabbits were killed 1 week after the last injection. The patellar tendons were harvested and examined using hematoxylin and eosin staining and transmission electron microscopy.

Results: Compared with the control groups, tendons exposed to PGE$_2$ by injection showed focal areas of hypercellularity, loss of normal tissue architecture, and focal areas of tendon disorganization and degeneration. Tendons injected with PGE$_2$ exhibited loosely organized collagen fibrils and had thinner collagen fibril diameter compared with control tendons ($P < 0.005$). Tendons injected with 500 ng PGE$_2$ appeared to be more disorganized and degenerated than those injected with 50 ng PGE$_2$.

Conclusions: Repeated exposure of the tendon to PGE$_2$ leads to degenerative changes within the tendon.

Clinical Relevance: It is known that human tendon fibroblasts produce PGE$_2$ in vitro and in vivo in response to repetitive mechanical loading. This study demonstrates that repetitive exposure of the tendon to PGE$_2$ can result in degenerative changes within the tendon. Therefore, PGE$_2$ produced by tendon fibroblasts in response to repetitive mechanical loading in vivo might contribute to the development of exercise-induced tendinopathy.

Key Words: prostaglandin-E$_2$, patellar tendon, tendinopathy, inflammation, degeneration (Clin J Sport Med 2005;15:27–33)

Tendon pathologic changes due to repetitive motion are frequently encountered clinical problems. The term tendinopathy is used to collectively refer to this group of tendon disorders. Despite the high prevalence of tendinopathies, their pathogenesis is poorly understood. Although repetitive mechanical loading is thought to be important in the development of tendinopathy, the cellular and molecular mechanisms underlying the tendon disorders remain poorly understood.

A few studies have been performed to investigate the pathophysiology of tendinitis (see review). These studies seem to suggest that repetitive mechanical loading leads to microscopic degenerative changes in tendon and paratenon. However, these studies do not address the developmental mechanisms of tendinopathy at the cellular and molecular levels.

There have been efforts to develop animal models for the study of tendinopathy. One such model showed that injection of bacterial collagenase into a tendon caused tendon injury. This collagenase injection model appeared to mimic a tendon healing response after injury. However, tendinopathy is an insidious process, and in many cases, normal tendon healing is impaired. Another model used a combination of inflammatory cytokines and other unknown factors (known as cell activating factor (CAF)). With this model, it was found that the injection of CAF increased cellularity at and around the injection site and decreased failure loads of the patellar tendons. However, because CAF is ill-defined and not produced by tendon fibroblasts under mechanical loading, it is not clear from this model whether any of these factors might be responsible for the development of tendinopathy.

In vitro studies have shown that repetitive mechanical loading of human tendon fibroblasts increases the production of prostaglandin-E$_2$ (PGE$_2$). PGE$_2$ is synthesized from arachidonic acid via the action of cyclooxygenase (COX), and...
mechanical stretching of human tendon fibroblasts has been shown to lead to increased COX expression levels. The in vitro finding appears consistent with that of an in vivo study showing that the levels of PGE2 were increased by 100% in the peritendinous space after exercise. Therefore, the production of PGE2 by fibroblasts in tendon, paratendon, or surrounding connective tissue in response to mechanical loading, as shown in in vitro and in vivo studies, might represent an important step in the development of tendinopathy. Indeed, a previous study has shown that peritendinous injection of prostaglandin-E1 around the rat Achilles tendon leads to degenerative as well as inflammatory changes around and within the tendon. The study suggested that prostaglandins might induce degenerative changes in tendons. Since tendon fibroblasts produce PGE2 in response to repetitive mechanical loading, the next logical step is to determine the in vivo effects of repetitive exposure of the tendon to PGE2. Given the findings of the aforementioned studies, we hypothesized that repeated exposure of tendons to PGE2 would result in degenerative changes similar to those seen in tendinopathy. To test the hypothesis, PGE2 was injected into rabbit patellar tendons at weekly intervals. The repeated injections were performed because we wanted to determine how a tendon responds to repeated exposure to PGE2 in vivo, and also because PGE2 has a short half-life, and therefore repeated injections increase the likelihood that the tendon tissue would be exposed to it. At the end of the experiment, hematoxylin and eosin (H&E) staining was performed on tendon sections, and the histologic changes between the experimental and control groups were compared. In addition, transmission electron microscopy (TEM) was used to examine collagen fibril organization and diameters obtained from the 2 groups.

MATERIALS AND METHODS

Ten skeletally mature female New Zealand White rabbits (weight range, 4.3–5.7 kg) were used in this study. The animal protocol was approved by the University of Pittsburgh Institutional Review Board (protocol #0108479). Briefly, the experiments were designed as following. For each rabbit, the right patellar tendon was designated as the experimental tendon, whereas the left patellar tendon was used as a control (Table 1). The experimental tendon received either 50 ng or 500 ng PGE2. The control tendon received 1 of the 3 treatments: no injection, or saline injection, or needlestick only. Four injections, 1 week apart, were administered. Before each injection, the rabbits were sedated by an intramuscular injection of ketamine (40 mg/kg) and xylazine (7.0 mg/kg). The skin over the patellar tendon was carefully shaved and sterilized with alcohol and iodine. The knee was firmly held in flexion at 90° to make the patellar tendon taut and prominent so that it could be palpated easily. A point halfway between the inferior margin of the patella, and the tibial insertion was identified as the target injection site. Using these landmarks, PGE2 (99% purity; Sigma-Aldrich Co, St. Louis, MO) was then carefully injected transcutaneously into the midsubstance of the patellar tendon (either 50 ng or 500 ng in 0.9% saline, total volume = 200 µL; n = 5 each) using a 26G syringe needle (Fig. 1). Again, the contralateral patellar tendons were used as 3 different controls: no injection (n = 2), 200 µL saline injection (n = 6), and needlestick only (n = 2). All the injections were performed by 1 experienced orthopedic surgeon to ensure that the site and manner of the injections were consistent. After injection, the animals were carefully monitored to ensure normal eating and ambulatory activity. The injection was repeated on days 7, 14, and 21. No weight loss or infection in the rabbits was noted, and no rabbits died during the experiments. The rabbits were killed on day 28 by an intracardiac injection of

TABLE 1. Scheme of Injection Regimen in Rabbits

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Right Patellar Tendon</th>
<th>Left Patellar Tendon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50 ng PGE2</td>
<td>Normal saline</td>
</tr>
<tr>
<td>2</td>
<td>50 ng PGE2</td>
<td>Normal saline</td>
</tr>
<tr>
<td>3</td>
<td>50 ng PGE2</td>
<td>Normal saline</td>
</tr>
<tr>
<td>4</td>
<td>50 ng PGE2</td>
<td>Needlestick only</td>
</tr>
<tr>
<td>5</td>
<td>50 ng PGE2</td>
<td>No injection</td>
</tr>
<tr>
<td>6</td>
<td>500 ng PGE2</td>
<td>Normal saline</td>
</tr>
<tr>
<td>7</td>
<td>500 ng PGE2</td>
<td>Normal saline</td>
</tr>
<tr>
<td>8</td>
<td>500 ng PGE2</td>
<td>Normal saline</td>
</tr>
<tr>
<td>9</td>
<td>500 ng PGE2</td>
<td>Needlestick only</td>
</tr>
<tr>
<td>10</td>
<td>500 ng PGE2</td>
<td>No injection</td>
</tr>
</tbody>
</table>

FIGURE 1. Illustration of the injection method used in this study. The knee was held in a flexed position at 90°, and using tibial insertion as a landmark, the tendon was injected into the midsubstance of the tendon along its longitudinal direction (dashed arrow).
phenobarbital, and the patellar tendons were harvested as follows. A superficial incision was made over the knee while it was being held at approximately 90° of flexion. The skin was gently reflected to expose the underlying patellar tendon, easily identified due to its shiny appearance. The proximal and distal attachments of the tendon were then identified. Using a sharp #10 scalpel, the tendon was first detached from the tibial insertion site and then lifted up to reveal its attachment to the patella. The distal end of the tendon was gently held with forceps, and a transverse incision along the inferior border of the patella was made to free it completely.

After harvest, the patellar tendons were immediately fixed in 10% formalin, embedded in paraffin, sectioned, and then stained with H&E. The stained tendon sections were examined at 2 different magnifications (×10 and ×20) using a light microscope (model #TE-DH100W; Nikon). Photographs were obtained using a digital camera (model #221; Diagnostic Instruments, Fredericksburg, VA) attached to the microscope assembly.

Transmission electron microscopy was also performed to examine collagen fibril organization and diameter. The tendon samples were prepared as follows. The tendon pieces were fixed in a solution containing 2% paraformaldehyde and 2.5% glutaraldehyde. They were then rinsed several times with 0.1% cacodylate buffer. The tendons were placed in a 1% aqueous solution of osmium tetroxide, followed by a series of rinses with ethanol solutions of increasing strength (50%, 70%, 95%, and 100%). Next, the tendons were placed for 1 hour in a 2:1 mixture of propylene oxide and epon, followed by an additional 1 hour in a 1:2 mixture of propylene oxide and epon. Finally, the tendons were embedded in pure epon, sectioned at 1-µm thickness with an ultra microtome, and stained with toluidine blue. A transmission electron microscope (model EM208-S; Philips) was then used to examine the tendon sections. To minimize possible biases in preparing tendon samples, experienced pathologists who were blind to the treatment protocol performed H&E staining and TEM analysis. Image analysis software (Scion Corp., Frederick, MD) was used to measure the diameter of collagen fibrils. An unpaired Student t test was used to determine whether the diameter of collagen fibrils from the PGE2-injected tendons was significantly different from that of control tendons. The difference was considered to be significant if P value was <0.05.

RESULTS

Light microscopy of H&E stained tendon samples showed that tendons injected with 50 ng PGE2 had focal areas of degeneration that were present both within and around the tendon (Fig. 2A). Fatty infiltration was noted in the tendon surrounded by highly disorganized tissue (Fig. 2B). There was a focal area of increased cellularity, but the matrix appeared to be regular in appearance (Fig. 2C). Tendons injected with 500 ng exhibited similar changes in histology, but overall they ap-
peared much more degenerated than those injected with 50 ng PGE₂ (Fig. 2D). The loss of parallel collagen fiber organization in the PGE₂-treated tendons was evident, and the presence of a large number of cells of unknown origin was noted within the tendons. In contrast, tendons from the 3 different control groups (no injection, normal saline injection, and needlestick only) did not show apparent inflammatory or degenerative changes (Fig. 2E), perhaps because the initial inflammation caused by the insertion of the needle had resolved by the time the rabbits were killed (1 week after the final injection). These tendons had a uniform appearance, with fibroblasts arranged in parallel. The fibroblasts had thin, flattened nuclei. The extracellular matrix was regular, without obvious degenerative changes, and there were no inflammatory cells present. Overall, the control tendons appeared to be tightly packed and highly organized.

Transmission electron microscopy analysis of tendons injected with 50 ng PGE₂ showed that the diameter of collagen fibrils appeared thinner than that of the controls, and the space between the fibrils was large and irregular (Fig. 3A). Tendons injected with 500 ng PGE₂ appeared to show more loosely packed collagen fibrils than those injected with 50 ng PGE₂ (Fig. 3B). For the control tendons that were given a needlestick only, TEM revealed that the collagen fibrils were tightly packed, with thick and thin fibrils organized in such a way that there was little unfilled space between them (Fig. 3C). Tendons injected with normal saline only showed a similar tightly packed appearance (Fig. 3D). The relative frequency distribu-

**FIGURE 3.** Effect of intratendinous injection of PGE₂ on collagen fibril organization. It is apparent that collagen fibrils of tendons injected with 50 ng PGE₂ (A) or 500 ng PGE₂ (B) were more disorganized compared with tendons that received needlestick only (C) or saline injection (D) (TEM, original magnification ×8000).
tion of collagen fibrils showed that overall tendons injected with PGE2 had a smaller fibril diameter compared with those injected with saline only (Fig. 4A). The collagen fibril diameter (mean ± SD) of tendons injected with 50 ng and 500 ng PGE2 was 16.06 ± 4.76 pixels and 15.65 ± 5.33 pixels, respectively, compared with 21.6 ± 5.51 pixels for the saline-injected control group (P < 0.005 for both the experimental groups vs. the control group; Fig. 4B).

DISCUSSION

This study demonstrates that repeated exposure of the tendon to PGE2 by injection led to a predominant pattern of degeneration in the tendon matrix. The repetitive exposure of the tendon to PGE2 caused disorganization of the collagen matrix as well as decreased diameter of collagen fibrils. Thus, the results support our working hypothesis that repetitive exposure of the tendon to PGE2 results in degenerative changes in tendon matrix. It is interesting to note that although PGE2 is a known inflammatory mediator, the absence of inflammatory cells in the tendon 1 week after repeated PGE2 injections suggests that the long-term effect of PGE2 on tendons may be degeneration instead of lasting inflammation. Furthermore, this study shows that repeated exposure of the tendon to PGE2 leads to decreased collagen fibrils diameter. Also, since PGE2 increases collagenase production by rabbit uterine cervical fibroblasts,15 it is possible that tendon fibroblasts that were exposed to PGE2 in vivo could produce collagenase, which may result in degenerative changes in rabbit tendons. Taken together, the results of this study suggest that PGE2, a known inflammatory mediator, might potentially be involved in ten-

![FIGURE 4. Relative frequency distributions of collagen fibril diameters with and without PGE2 injection are shown (A). The diameter of collagen fibrils of tendons with injection of PGE2 was smaller than that of tendons without PGE2 injection. Specifically, for the 50 ng and 500 ng injection groups, the collagen fibril diameter (mean ± SD) was 16.06 ± 4.76 pixels and 15.65 ± 5.33 pixels, respectively, compared with 21.6 ± 5.51 pixels in the saline-injected group (B).](image-url)
tendon degenerative changes in vivo. An in vivo microdialysis study has shown that levels of PGE\textsubscript{2} were consistently increased by 100\% in the Achilles peritendinous space after exercise in normal subjects; however, the small sample size (6 subjects) in the study might be a reason that this difference was not statistically different.\textsuperscript{12} Another in vivo microdialysis study in Achilles tendons with chronic tendinopathy showed consistently increased PGE\textsubscript{2} levels, but the number of subjects in the study was quite small (4 pathologic tendons, 5 control tendons).\textsuperscript{16}

It is well recognized that the most common histologic appearance of tendons with tendinopathy is characterized by degeneration and focal necrosis without apparent inflammatory changes.\textsuperscript{17–19} Biopsies from patients with tendinopathy demonstrate that these changes most often involve a focal segment of the tendon.\textsuperscript{20} Less commonly, microscopic tears, hemorrhage, and cellular infiltrates are also seen.\textsuperscript{21} It should be noted that special staining techniques have shown that the cells seen within tendinopathic tendons are not of inflammatory origin, but rather are myofibroblasts.\textsuperscript{18} We speculate that after initial inflammation, an active reparative process by myofibroblasts takes place in the tendon. Overall, our findings are largely consistent with the known features of tendinopathy found in these previous studies, and therefore injection of PGE\textsubscript{2} into the tendon may be used as a model of tendinopathy. It should be added that degeneration seen in our PGE\textsubscript{2}-injected tendons was unlikely due to the method of delivery, since the histology of saline-injected tendons was not different from that of needle-stuck tendons. Furthermore, the higher dose of PGE\textsubscript{2} appeared to associate with a more marked degeneration in tendons, although the possibility of supraphysiologic and toxic effects from the 500-ng dosage of PGE\textsubscript{2} could not be excluded. Note that there was no significant difference in collagen fiber diameters between the 2 dosages of PGE\textsubscript{2} used in this study. The reason for this may be that a low dosage of PGE\textsubscript{2} (50 ng) saturates PGE\textsubscript{2} receptors,\textsuperscript{22} so that a higher dosage of PGE\textsubscript{2} (500 ng) no longer affects collagen fiber diameters. The differences in the dosage effect of PGE\textsubscript{2} on tendon degeneration and collagen fiber diameter also suggest that the detailed molecular mechanisms for these two events of extracellular matrix repairing/remodeling are different.

Note that this study measured collagen fiber diameter to assess the influence of exposure of PGE\textsubscript{2} on tendon. Smaller than normal-diameter collagen fibrils are related to the weakness of scar tissue in ligaments.\textsuperscript{23} Therefore, small collagen fibers due to the exposure of tendon to PGE\textsubscript{2} may potentially result in decreased mechanical strength of the tendon, which could make it susceptible to rupture.\textsuperscript{5}

There are a few limitations of this study. First, since the observation time was relatively short (28 days), the long-term effects of PGE\textsubscript{2} exposure on the tendon could not be assessed. Second, earlier acute events following PGE\textsubscript{2} injection were likely missed, as was the identity of the hypercellular regions.

Third, it is not known what effects different regimens of PGE\textsubscript{2} exposure by injection (eg, weekly versus biweekly injection) would have had on the histologic properties of the tendon. Finally, biomechanical properties of the PGE\textsubscript{2}-exposed tendons were not investigated in the present study. Future studies should examine the comprehensive effect of PGE\textsubscript{1}, on the histologic, biochemical, and biomechanical properties of the tendon. Also, future investigations into tendinopathy in animal models should use noninvasive technologies, such as ultrasound and MRI, to examine inflammatory and/or degenerative changes of tendon with time. Both ultrasound and MRI have been clinically used for diagnosis of tendinopathy.\textsuperscript{24} The usage of these imaging techniques in animal studies of tendinopathy can provide insights into the developmental process of tendinopathy. Finally, the efficacy of different treatments for tendinopathy, such as NSAIDs, corticosteroids, and shock-wave therapy,\textsuperscript{25} may be evaluated on this model of tendinopathy.

**ACKNOWLEDGMENT**

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**REFERENCES**


