A Rabbit Model of Collagenase-Induced Patellar Tendon Degeneration: Effect of Single versus Repeated Collagenase Injections

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Disclosures:

Introduction: Chronic degenerative tendinopathy is a common clinical condition that results in pain, disability, and significant healthcare costs [1]. Histologic evaluation of end-stage degenerative tendons often reveals collagen matrix disruption, tenocyte hypercellularity, neovascularization, and fatty infiltration, often without any evidence of inflammation [2]. Although repetitive local microtrauma is thought to be the primary etiologic factor, the pathogenesis of this disease is still unclear. Thus, preclinical models of tendinopathy are needed in order to better understand chronic tendon pathologies and to assess the efficacy of novel treatments. Researchers have attempted to develop rodent models of tendon degeneration by subjecting the tendons to mechanical overuse [3], but this strategy has limited utility for larger species such as the rabbit. Collagenase injections into the rabbit patellar tendon have been used to disrupt the tendon matrix and produce short-term degenerative changes [4], but it is unclear if aspects of tendon degeneration persist for longer time periods. In this study, we examined the histological and biomechanical effects of single versus repeated collagenase injections in a rabbit model of collagenase-induced patellar tendon degeneration at two different time points (4 and 16 weeks post-injection). We hypothesized that repeated collagenase injections would cause degenerative changes to the patellar tendon that would persist for at least 16 weeks.

Methods: Skeletally mature female NZW rabbits (age 1.0 - 2.5 years) were used in this study. Since rabbits of different ages were used, we first verified that there were no age-related changes in normal patellar tendon biomechanical properties (data not shown). Patellar tendons received either no injection (‘normal’ group, n = 7), a single injection of bacterial collagenase (200IU in 0.05 ml saline) into the distal insertion (‘200IU’ group, n = 10), or an initial 200IU collagenase injection followed 4 weeks later by an identical 200IU collagenase injection in the same location (‘200IU + 200IU’ group, n = 14). Healing tendons were harvested at either 4 or 16 weeks following the last injection for histology (n = 1-2 per time point) and biomechanics (n = 3-7 per time point). Histologic sections were stained with hematoxylin and eosin for qualitative analysis. For biomechanical analysis, the patellar tendon-bone units were dissected away from surrounding structures and the tendon’s width was cut down to its central third. Tendon dimensions were measured with calipers and Verhoeff’s stain lines were applied to the anterior surface for optical strain measurement. Each specimen was submerged in 0.9% PBS at 37°C, preconditioned between 1.2 and 5.0% strain at 0.5 Hz for 50 cycles, and then failed in uniaxial tension at a strain rate of 20%/sec. Using video (30 fps) recorded during the failure test, regional tissue strains were calculated by optically tracking the applied strain lines. At each time-point, significance between treatment groups (p < 0.05) was assessed using one-way ANOVA followed by Fisher’s least significant difference (LSD) post-hoc comparisons.

Results: Single collagenase injections: Single 200IU injections of collagenase elicited severe matrix disorganization, hypercellularity, nuclear hypertrophy, and neovascularization at 4 weeks. At 16 weeks, the tendon still appeared hypercellular but the matrix had realigned along the axis of tension (Fig. 1). In accordance with the histologic results, biomechanical properties were significantly reduced at 4 weeks, but no statistically significant biomechanical differences were found between the 200IU group and the normal group at 16 weeks (data not shown). Repeated collagenase injections: In contrast to a single injection, repeated injections of 200IU collagenase resulted in statistically significant reductions in structural and material properties at both 4 and 16 weeks (Table 1). There was a significant increase in biomechanical properties from the 4 week time point to the 16 week time point, indicating that healing was actively occurring over this time period. Regional strain analysis at 16 weeks revealed decreased surface strains at the site of injection (the tibial insertion) compared to normal tendon (Fig. 2), likely as a result of extensive fibrosis and scar formation in this region. Interestingly, local strains were increased in the proximal midsubstance, possibly due to alterations in loading as a result of matrix disruption.

Discussion: In this study, we have shown that repeated collagenase injections into the rabbit patellar tendon provide a simple and reproducible injury model that recapitulates many of the histologic and biomechanical features of tendon degeneration for up to 16 weeks. Such a model will be useful for studying the natural progression of chronic tendon pathologies and for evaluating potential treatments for degenerative tendinopathy. Limitations of this study include small sample sizes and only two evaluation time points, thus it is unknown how long the biomechanical deficits actually persist in this model or if the tendons ever recover normal biomechanical properties. Future work will include more detailed histologic analysis in order to better assess any regional differences (e.g. insertion site versus midsubstance) in degenerative response and to more accurately compare the features of collagenase-induced degeneration in this model to other animal models of tendinopathy.
Significance: Chronic tendinopathy is an important clinical problem that is not well understood. New animal models that accurately replicate the hallmark features of tendon degeneration are needed in order to study the etiology, pathogenesis, and treatment of this debilitating condition.

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Table 1. Structural and material properties of patellar tendons injected with repeated 200IU doses of collagenase (mean ± standard deviation).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Ultimate Load (N)</th>
<th>Stiffness (N/mm)</th>
<th>Ultimate Stress (MPa)</th>
<th>Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal patellar tendon</td>
<td>465.7 ± 69.1</td>
<td>237.0 ± 34.6</td>
<td>105.4 ± 15.4</td>
<td>1184.9 ± 70.3</td>
</tr>
<tr>
<td>200IU + 200IU, 4 wks (n = 7)</td>
<td>127.8 ± 26.8*</td>
<td>75.7 ± 15.8*</td>
<td>16.0 ± 4.7*</td>
<td>251.9 ± 64.5*</td>
</tr>
<tr>
<td>200IU + 200IU, 16 wks (n = 5)</td>
<td>234.4 ± 61.2**</td>
<td>147.0 ± 26.7**</td>
<td>35.6 ± 12.2**</td>
<td>541.4 ± 95.8**</td>
</tr>
</tbody>
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* Significantly different compared to normal patellar tendon.
** Significantly different compared to 200IU + 200IU at 4 weeks.

2004.
Figure 1. Rabbit patellar tendons injected with 200IU collagenase at 4 weeks (A) and 16 weeks (B) compared to normal, healthy tendon (C).
Figure 2. Regional strain patterns in normal and collagenase-treated tendons.