Elastase and Tendinopathy: Their Clinical Connection, and a Novel Rat Model of Elastase-Induced Chronic Tendinopathy

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Introduction: Typical histological changes in tendinopathy include hypervascularization, a reduced number of cells, and matrix degeneration in most components (collagen, proteoglycans, etc.). Mild and moderate matrix degeneration in tendinopathy characteristically shows collagen arrangements with decreased collagen I and increased collagen III. Elastic fibers are also present in the tendon and have an important mechanical function, but their role in the complex mechanisms of the pathogenesis of chronic tendinopathy is not understood. We sought to determine the effects of elastin and the elastin-related cytokines present in clinical tendinopathy to determine the role of elastin in this common disorder. We also investigated the effects of peritendinous injections of elastase on the Achilles tendons of rats, and we developed a new animal model of chronic tendinopathy.

Methods: We examined biopsy specimens and typical magnetic resonance image findings in those specimens from the biceps or rotator cuff tendon in twelve patients clinically diagnosed with rotator cuff tendinopathy. The presence of elastase, an elastin-related cytokine, was examined using immunohistochemical staining and was correlated with the histological grading of the tendinopathy. Ultrasonographically-guided peritendinous injections of elastase into the Achilles tendons of 90 rats were used to create an experimental model of chronic tendinopathy. The experimental group (n = 60) was injected with elastase (1 U/20 μl). The control group (n = 30) was injected with the same volume of phosphate-buffered saline (PBS). Ultrasonographic and functional evaluations of automated static weight-bearing were measured using an incapacitance tester every week for 56 days.

Results: Results from clinical specimens showed that the percentages of elastase-positive cells (Fig. 1) per unit area of grade II extracellular matrix (ECM) structure (96.5% ± 1.7) and grade III ECM structure (95.7% ± 3.3) were significantly higher than for grade I ECM structure (26.9% ± 10.02) (P < 0.001). Thus, we used elastase to create an animal model of tendinopathy based on our clinical findings. In our animal model, the thickness of the tendon significantly increased from 21 to 56 days (Fig. 2A, B, C), and dynamic weight bearing ratio of left to right was decreased from 42 days after the elastase injection (Fig. 2D). The number of cells and histological changes were detected using H&E staining (Fig. 3A, C, E, G). Hypercellularity, hypervascularity, and focal lesions appeared from 28 days post-injection of elastase. Masson trichrome staining was used to detected collagen components (Fig. 3B, D, F, H). Severely degraded collagen structures with a total loss of fiber orientation and severely fragmented fibers appeared from 28 days post-injection. Elastase expression markedly increased from 7 days until 56 days, but elastin levels continuously decreased until 56 days post-injection (Fig 4A, C, D). Consistent with our histological results, Western blotting and immunohistochemical staining showed that collagen I expression decreased from 28 days, but collagen III expression increased (Fig. 4A, B, E, F).

Discussion: These experiments extend knowledge of human tendon elastin and its degradation by elastase in chronic tendinopathy. This novel animal model provides high reliability and reproducibility for creating a tendinopathy that is very similar to human chronic tendinopathy. It also highlights a research potential in the study of the pathogenesis of chronic tendinopathy, its mechanisms, and possible treatment interventions.

Significance: This is the first clinical and in vitro study to show that higher elastase expression is another feature of tendinopathy. Our findings suggest that elastase might be important in chronic tendinopathy. Our animal model shows consistent and characteristic changes in chronic tendinopathy and reflects the pathophysiological processes during the development of this disorder.

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Figure 1. Immunohistochemical staining of elastase in tendinopathy samples. A, Normal control tissue shows weak elastase expression; B, Elastase expression is increased in tendinopathy; C, High magnification of elastase expression in tendinopathy; D, Bar graph showing the positive cell rate of elastase in different grades of tendinopathy. P<0.001.
Figure 1. Histograms showing nuclear numbers at 0, 1, 7, 14, 28, 56 days after injection of elastase.

A-C: Examples of histological changes observed at 0 days, 14 days, 28 days, and 56 days after injection of elastase.

Bar graphs show the comparison of nuclear numbers at different time points.

***Significant difference (p < 0.001) compared to the control group.
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[Image of scientific figures and graphs]

Figure X: Protein expression analysis for Collagen I and Collagen III over time. A) Western blotting for Collagen I and Collagen III. B) Stained sections for Collagen I and Collagen III. C) Quantitative analysis of Western blotting data. D) Stained sections for Collagen I and Collagen III. E) Western blotting for Collagen I and Collagen III. F) Stained sections for Collagen I and Collagen III.