THE EFFECT OF AMPLITUDE AND FREQUENCY OF CYCLIC TENSILE STRAIN ON THE INHIBITION OF MMP-1 EXPRESSION IN RAT TAIL TENDON CELLS

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INTRODUCTION

Physical stress plays a critical role in the homeostasis of connective tissues. Previous work in our laboratory has demonstrated the deleterious effects of immobilization on the material properties of tendons and the reversal of these effects with cyclic loading [1]. While the exact mechanism by which stress-levels determine tissue homeostasis is still unclear, several investigators have implicated the role of interstitial collagenase (MMP-1) in this process [2,3]. Other studies from our lab have shown that tendon cells respond to tensile load through a cytoskeletally-based mechanotransduction system [4]. It is possible that cyclic tensile strain may play an important role in maintaining tissue homeostasis through a MMP-1 inhibitory mechanism in tendon cells. Therefore, it was the purpose of this study to examine the effect of cyclic strain and the role of the cytoskeleton on the inhibition of MMP-1 expression in rat tail tendon cells. It was our hypothesis that cyclic strain would inhibit MMP-1 expression in a dose dependent (magnitude and frequency) manner through a cytoskeletally-based mechanotransduction system.

METHODS

To determine the effect of various cyclic strain regimes on the expression of MMP-1 in tendon cells, Sprague-Dawley rat tail tendons were cyclically displaced at 1, 3, or 6% strain at 0.017Hz or at 1% strain at 0.017, 0.17, or 1.0Hz for 24 hours. To determine the role of the cell cytoskeleton in MMP-1 gene expression, rat tail tendons were cyclically displaced at 6% strain at 0.017Hz with and without 10μM cytochalasin D, an actin depolymerizer. Cyclic strain was applied to tendons using a custom, computer-controlled, stepper motor driven device (Figure 1). Tendons were placed in the device until all visible slack was removed to approximate 0% strain and then clamped in the grips to prevent slipping. Northern blot analysis was used to assay for mRNA expression of MMP-1 in stress-deprived as well as strained tendons. There were twenty tendons per regime and each experiment was repeated three times. To verify the *in situ* disruption of the actin cytoskeleton by cytochalasin D, additional rat tail tendons were prepared for actin staining and confocal laser microscopy (Figure 2).

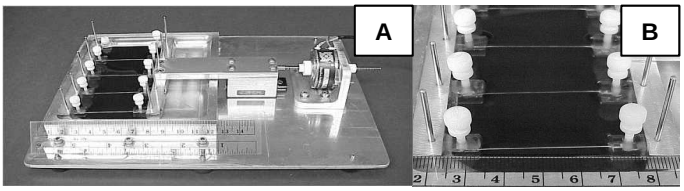

Figure 1. A: The computer-controlled, stepper motor driven, cyclic loading system. B: Close up view of the testing system showing rat tail tendons in place.

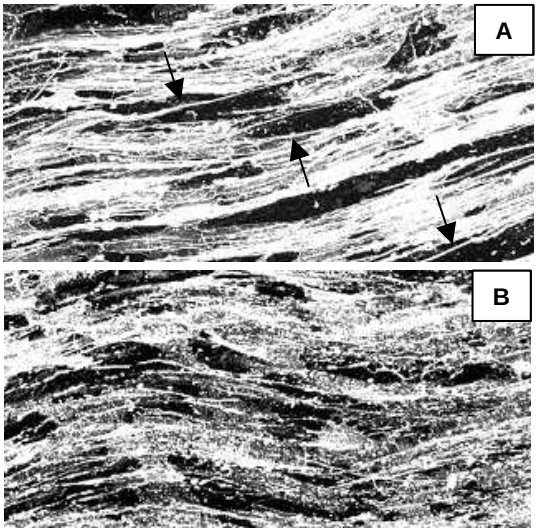

Figure 2. Confocal overlay images of rat tail tendon stained with rhodamine phalloidin to label actin fibers. A) Fresh tendon: note presence of organized actin stress fibers (arrows). B) Tendon treated with 10μM cytochalasin D for 1hr: note there is a loss of actin fiber organization. (confocal 40x oil immersion)

RESULTS

The results of this study show that a low level of strain (1%@0.017Hz) significantly inhibits, but does not totally eliminate MMP-1 expression when compared to immobilized controls (Figures 3 and 4). However, increasing the strain level (3%, 6%) or frequency (0.17Hz, 1.0Hz) completely eliminated MMP-1 expression (Figures 3 and 4). The addition of cytochalasin D completely abrogated the inhibitory effect of cyclic loading on MMP-1 mRNA expression (Figure 5).

Figure 3. Northern Blot of MMP-1 mRNA expression from rat tail tendons cyclically strained at 0.017Hz at varying amplitudes for 24 hours.

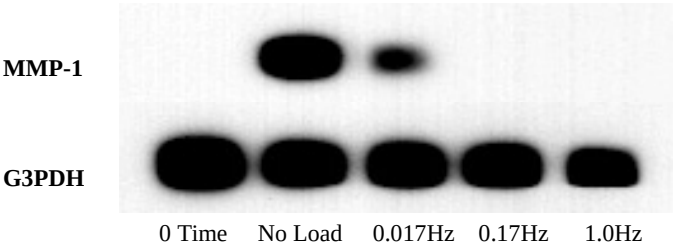

Figure 4. Northern Blot of MMP-1 mRNA expression from rat tail tendons cyclically strained at 6% strain at varying frequencies for 24 hours.

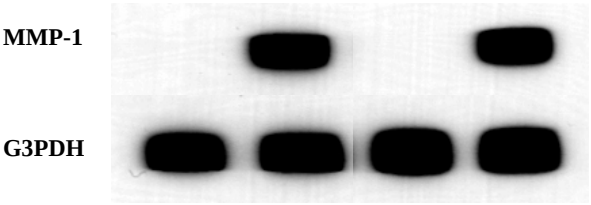

Figure 5. Northern Blot of MMP-1 mRNA expression from rat tail tendons cyclically strained at 6% strain at 0.017Hz with and without Cytochalasin D for 24 hours.

DISCUSSION

The results of this study suggest that MMP-1 expression, in tendon cells, responds to increases in amplitude and frequency of cyclic tensile strain in a dose dependent manner. A low level of strain (1%@0.017Hz) significantly inhibits MMP-1 mRNA expression and by increasing strain amplitude (3%, 6%) or frequency (0.17Hz, 1.0Hz) MMP-1 mRNA expression is eliminated. This amplitude inhibition of MMP-1 expression correlates with increased loss of crimp and cell nucleus deformation. The inhibition, but not elimination of MMP-1 expression at 1% strain may correlate with the number of cells whose cytoskeletons deformed enough to initiate the mechanotransduction response, as tendon crimp is still present at this strain. The inhibition of MMP-1 at higher frequencies may be a result of cytoskeleton deformation from increased shear strain caused by the fluid flow from increased loading rates. When the cellular actin cytoskeleton was disrupted through cytochalasin, it prevented the mechanotransduction process and thus abrogated the inhibitory effect of high amplitude stress on MMP-1 expression. Understanding the role of exercise on gene expression may help determine optimal exercise protocols for both injured and healthy tissues through the controlled application of load and frequency. This could lead to advances in overuse injury prevention and optimal rehabilitation protocols following tendon injury and repair.

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