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Mechanical and Histological, but not Functional, Properties Remain Inferior in Conservatively Treated Achilles Tendons in Rodents: Long Term Evaluation

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Introduction

Conservative treatment (non-operative) for Achilles tendon ruptures is suggested to produce equivalent capacity for return to function as operative repair[1]. In a rodent study, coupling conservative treatment with early return to activity (RTA) further improved healing and limb function 3- and 6-weeks post-injury[2,3]. However, the long-term biomechanical effects of conservative treatment and RTA timing on limb function, Achilles tendon properties, and gastrocnemius/soleus muscle properties are unknown and are essential to fully understand the effects of this treatment paradigm. Therefore, the purpose of this study was to evaluate the long-term response of conservatively treated Achilles tendons in rodents with varied RTA. We hypothesized that tendon and muscle properties would be superior with earlier RTA compared to delayed RTA, but both injured groups would remain inferior to uninjured controls 16-weeks post-injury. We also hypothesized that no differences in limb function would exist between groups by 16-weeks post-injury, but early RTA would have accelerated return to normal limb function.

Methods

Study Design

Sprague Dawley rats (n = 42) at 16-weeks of age were used (IACUC approved). Animals had treadmill training[2,3] prior to blunt midsubstance transection of the right Achilles tendon and resection of the plantaris longus tendon[4]. Animals were randomized into groups that returned to activity after 1-week (RTA1) or 3-weeks (RTA3), and all hind limbs were immobilized in plantarflexion. Uninjured age-matched animals (n = 10) allowed only cage activity were used as controls. Functional evaluation (n = 10-16/group) of passive ankle joint range of motion (ROM) and stiffness was performed on anesthetized animals prior to injury, as well as 4, 6, 10 and 16 weeks post-injury.

Ex vivo Assays

After sacrifice 16-weeks post-injury, tendons (n = 10/group) were harvested and prepared for viscoelastic, quasi-static, and fatigue testing, as described[2,3]. An additional set of tendons (n = 6/group) was used for histological and immunohistochemical analyses. Sagittal sections (7 μm) were stained with Hematoxylin-Eosin (H&E) and Safranin-O and Fast Green (SAF-O). Additionally, the gastrocnemius/soleus muscle complex was harvested, sectioned axially, and stained for antibodies against laminin (L9393, Sigma Aldrich) and myosin heavy chain (MyHC) types 1, 2a, and 2b (type 1: BA-D5; type 2a: SC-71; type 2b: BF-F3), as described[4].

Analysis

Functional ankle joint properties (i.e., ankle ROM and stiffness) for both dorsiflexion and plantarflexion were evaluated. Achilles tendon viscoelastic, quasi-static, and fatigue properties were computed. The tendon midsubstance was evaluated for cell density, nuclear shape, and GAG staining through grading by 3 independent, blinded investigators. Deep and superficial muscle regions were analyzed for fiber size (min Feret diameter) and fiber type distribution using the SMASH application[5]. One-way ANOVAs with post hoc t-tests with Bonferroni corrections were used to compare the effect of RTA on mechanical, functional, structural, and muscle properties. Kruskal-Wallis tests with post hoc Mann-Whitney U-tests were used for histological scoring.

Results

Functional deficits due to injury and due to RTA timing were present 4-weeks post-injury for ambulatory measures including the vertical ground reaction force (Fig. 1A) and for passive dorsiflexion ROM (not shown), both of which gradually returned to baseline levels by 16-weeks. Although functional properties achieved pre-injury levels by 16-weeks post-injury, mechanical properties remained inferior. RTA1 and RTA3 groups had increased cross sectional area (Fig. 2A) and decreased dynamic modulus (|E*|) (Fig. 2B) compared to uninjured control tendons. With regard to fatigue properties, the secant modulus (material property) was decreased in RTA1 and RTA3 groups compared to control (not shown), while the secant stiffness (structural

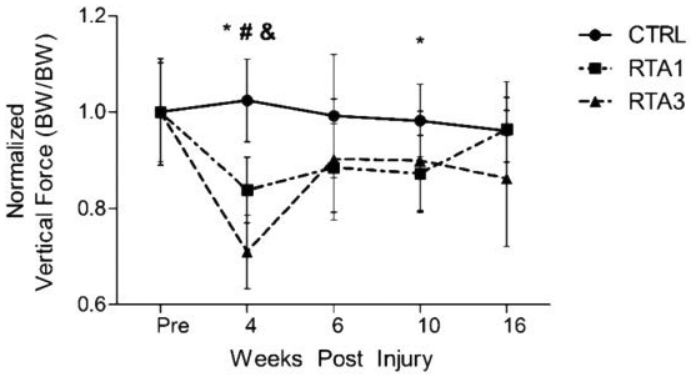

Figure 1. Ambulatory Assessment. RTA1 and RTA3 groups had decreased vertical force 4-weeks post-injury and returned to control levels by 6-weeks post-injury. Data shown as mean ± SD. Symbols indicate significant differences ($p < 0.017$) between groups (*-CTRL v. RTA1; #-CTRL v. RTA3, &-RTA1 v. RTA3).

property) was not different between groups (Fig.2C). In addition, tendon laxity (Fig.2D) was elevated in RTA1 and RTA3 groups compared to control levels. Histologically, RTA1 and RTA3 groups had increased cellularity compared to control tendons (Fig.3A), but had no differences in cell shape or SAF-O staining. Muscle staining revealed that RTA1 groups had decreased fraction of type-2x positive fibers compared to control and RTA3 groups, but no changes in the fraction of type-1, type-2a, or type-2b fibers (Fig.3B). No changes in fiber size were detected.

Discussion

This study investigated the long-term effects of conservative treatment on Achilles tendon healing in rodents. Long-term functional outcomes were improved compared to those

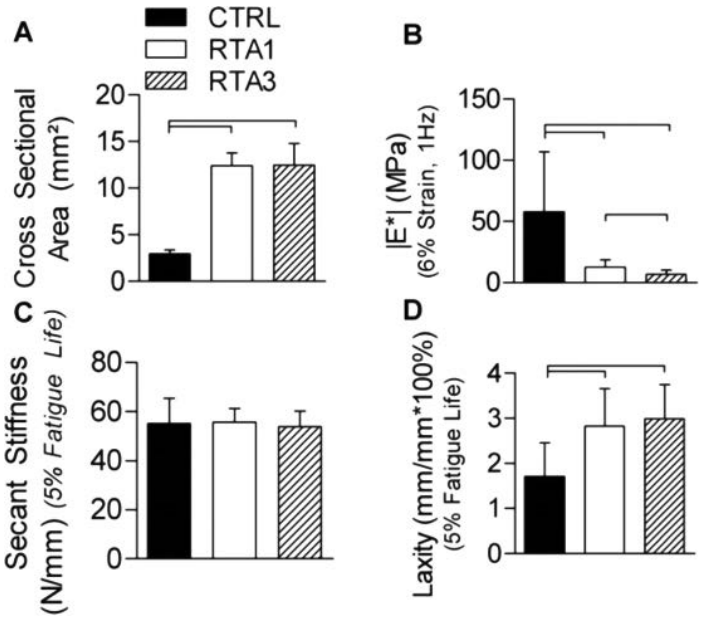

Figure 2. Mechanical Properties. RTA1 and RTA3 groups had increased **(A)** cross sectional area, decreased **(B)** IE*I, and increased **(D)** laxity compared to uninjured control tendons 16-weeks post injury. No differences in secant stiffness were found between groups. Data shown as mean ± SD. Lines indicate significant differences ($p < 0.017$).

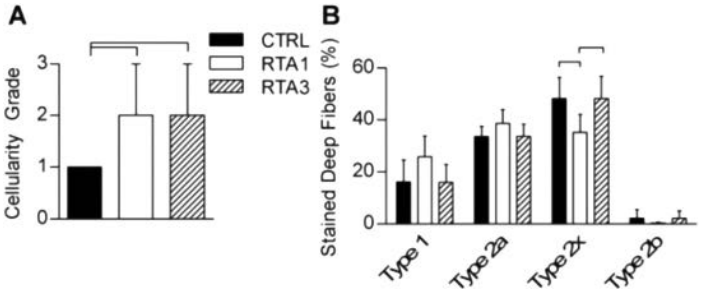

Figure 3: Tendon and Muscle Histological Properties. **(A)** Cellularity was increased in RTA1 and RTA3 tendons compared to uninjured control tendons when assessed at the midsubstance 16-weeks post-injury. **(B)** There was a lower percentage of type 2x fibers in RTA1 tendons compared to control and RTA3 groups. A- Data shown as median ± IQR. B- Data shown as mea n ± SD. Lines indicate significant differences ($p < 0.017$).

described in previous clinical literature[7, 8], potentially since an initial period of full plantarflexion casting was used to reduce tendon gapping, and that the function assessed was voluntary and not during high dynamic loading. Although mechanical properties improved compared to earlier time points in our rat model[2, 3] (similar to humans[6]), biomechanical properties at the tissue level remained significantly lower than controls. RTA1 and RTA3 groups displayed a much larger scar cross sectional area that was not fully remodeled, along with persistent high cell counts. Interestingly, although material properties were inferior in RTA1 and RTA3 tendons, structural properties were similar to controls, suggesting that healing tendons can achieve similar mechanical stiffness, which may explain the lack of changes in muscle properties between groups. Indeed, the majority of muscle properties assessed were not different from control levels 16-weeks post-injury, suggesting that conservative treatment does not have adverse effects on long-term muscle properties at the fiber level. The relatively low SAF-O staining contrasts to early RTA groups at 6-weeks post-injury[3], which suggests that a more tendon-like phenotype is achieved 16-weeks post-injury. Future studies will examine structural properties and sex differences in long term Achilles healing.

Significance

As conservative management of Achilles tendon ruptures becomes more popular, it is necessary to evaluate the long-term biomechanical effects of this treatment paradigm. Although functional properties return to baseline levels by 16-weeks in this rodent model, tendon properties remain altered mechanically and histologically, and gastrocnemius muscle properties may also be affected. Despite these changes, tendons 16-weeks post injury achieved many structural property characteristics (e.g., stiffness) of uninjured tendons, and differences created by prolonged casting were minimal.

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