Effect of Ultrasound-Guided Dry Needling on the Healthy Rat Supraspinatus Tendon

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Introduction

Chronic tendon injuries or overuse resulting in tendinopathy are common clinical problems. Conservative treatments, including rest, physical therapy and NSAID administration, are often ineffective. Recently, there has been an emergence of novel treatments for tendinopathy utilizing dry needling, or repeatedly introducing a needle into the abnormal tissue.[1,2] It is believed that micro-trauma to the tendon leads to disruption of pathological tissues, induction of bleeding, and release of factors to stimulate healing. Dry needling, typically done using ultrasound guidance, has been described alone as well as in combination with the injection of platelet-rich plasma, autologous blood,[3] glucose,[4] among other substances, demonstrating promising results, though no randomized controlled studies have been performed. Moreover, a controlled laboratory model to study this potential, as well as basic evidence supporting this practice is lacking.[5] Therefore, the objective of this study was to perform ultrasound-guided dry needling in healthy rat supraspinatus tendons to evaluate the acute vascular, biological, and mechanical response in the tendons, to understand the initial effects of dry needling. We hypothesized that there would be an early increase in blood flow, inflammation, cellularity, and matrix formation. Additionally, there would be a decrease in material properties, but no change in the structural properties of the needled tendon compared to healthy control tendon.

Methods

Study Design: 32 Sprague-Dawley rats were used (IACUC approved). 22 rats were subjected to bilateral ultrasound-guided dry needling and randomly assigned for sacrifice at days 1, 3 or 7 following the procedure to evaluate histology ($n = 6$ at days 1, 3, and 7) and mechanics ($n = 10$ at day 7). 10 healthy rats were sacrificed to serve as controls. 6 randomly assigned rats underwent color Doppler ultrasound imaging 24 hours prior to needling (as an internal control), 5 hours and 24 hours after needling.

Dry Needling: Using a 14 MHz ultrasound transducer, the rotator cuff was visualized in the transverse plane. A 27G needle was inserted posteriorly and guided between the humeral head and acromion to enter the supraspinatus tendon. The tendon was penetrated 10 times along its length. This technique was validated to consistently and accurately needle the rat supraspinatus tendon.

Color Doppler: Using a 14MHz transducer, B-mode and color Doppler images were acquired for each shoulder. A regional analysis of blood flow (between the humeral head and the skin) evaluated changes in the surrounding vasculature, and a local analysis (between the humeral head and the acromion) evaluated blood flow in the tendon region. The mean color level (MCL—average velocity of colored pixels), the fractional area (FA - percent of colored pixels in region of interest), and the color weighted fractional area (CWFA—average flow per total unit area of the tissue) were quantified. A one-way ANOVA with repeated measures and a Bonferroni post hoc test were performed ($^*p < 0.05$).

Mechanics: The right supraspinatus tendon was dissected and prepared for tensile mechanical testing with preconditioning, stress-relaxation, and ramp to failure as described.[6] Images were taken during testing to calculate 2D Lagrangian strain. T-tests were performed ($^*p < 0.05, \#p < 0.1$).

Histology and Immunohistochemistry: The left supraspinatus tendon was processed, embedded, sectioned at 7μm, and stained for safranin-o/fast green (Saf-O), hematoxylin and eosin (H&E), interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), and type III collagen. Images were graded by three blinded investigators for H&E cell shape (1-spindle to 3-round), H&E cellularity (1-low to 3-high), Saf-O staining intensity (1-low to 3-high), and DAB staining intensity (1-low to 4-high). A Kruskal-Wallis test and a Dunn's post hoc test were performed ($^*p < 0.05, \#p < 0.1$).

Results

For Doppler imaging, the CWFA in both the local and regional areas were significantly increased 5 hours-post dry needling (Figure 1). There was a significant increase in cross sectional area, and mechanical testing revealed a significant decrease in maximum stress, insertion modulus, midsubstance modulus, and stiffness (Figure 2). For histology, there was a significant

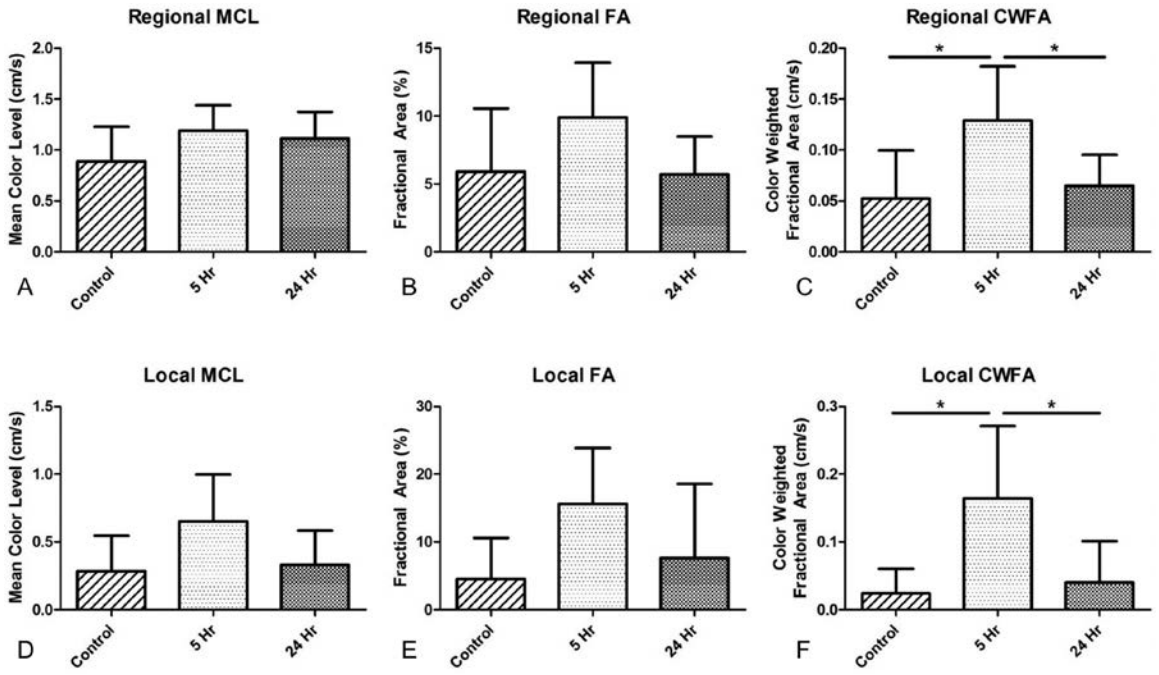

Figure 1. Regional (A-C) and local (D-E) analysis of color Doppler imaging. There was an increase in the color weighted fractional area (CWFA) 5 hours after the dry needling procedure in both regions (C, F). The mean color level (MCL) and fractional area (FA) were not significantly different at any time point. Data presented as mean± standard deviation.

increase in IL-1β, type III collagen, and rounded cell shape at day 1, a significant increase in cellularity at days 1 and 7, and a significant increase in glycosaminoglycans (GAG) content at days 3 and 7 (Figure 3).

Discussion

Dry needling the rat supraspinatus tendon caused an increase in blood flow, the initiation of an inflammatory response, and formation of granulation tissue. Doppler imaging showed increases in blood flow both regionally in the shoulder, as well as in the needled tendon area, supporting the induction of a systemic and local response to this micro-damage. Histology results support a cellular response to this micro-damage, with an early increase in cellularity with a more rounded cell shape. Granulation tissue was formed as indicated by the increases in type III collagen and GAGs. Additionally, the increase in inflammatory mediators is consistent with a previous study evaluating dry needling in injured rat tendons.[7] Mechanical properties confirm that micro-damage was induced in the tendon, initiating

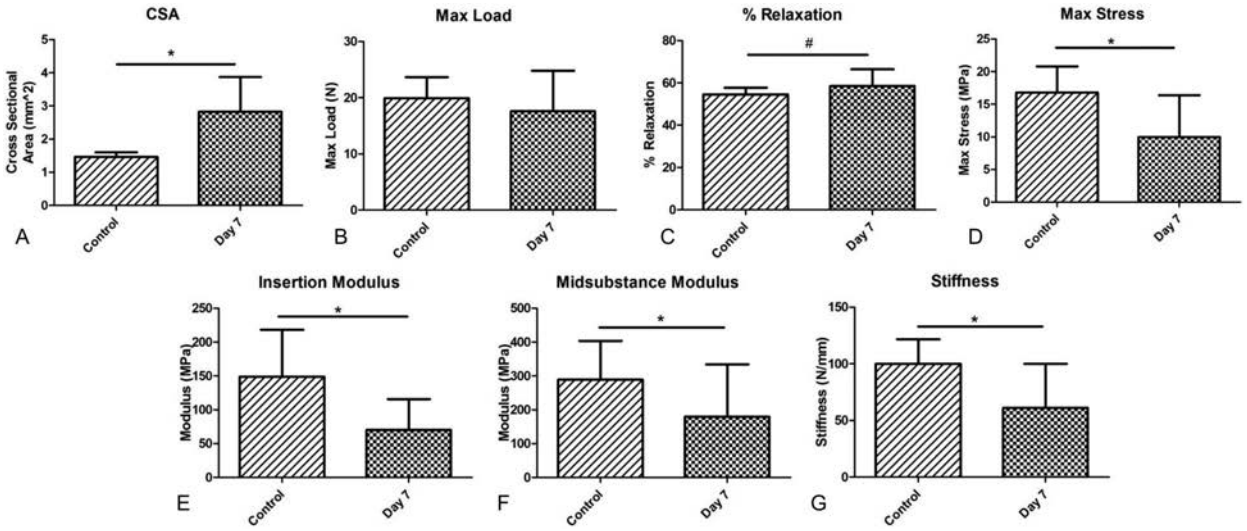

Figure 2. Mechanical properties of uninjured (control) supraspinatus tendons compared to dry needled tendons 7 days after the procedure (Day 7). The dry needled group showed a significant increase in cross sectional area (A) and a significant decrease in max stress (D), insertional and midsubstance modulus (E,F), and stiffness (G). There was a trend in percent relaxation (C) and no differences in max load (B) compared to control. Data presented as mean ± standard deviation.

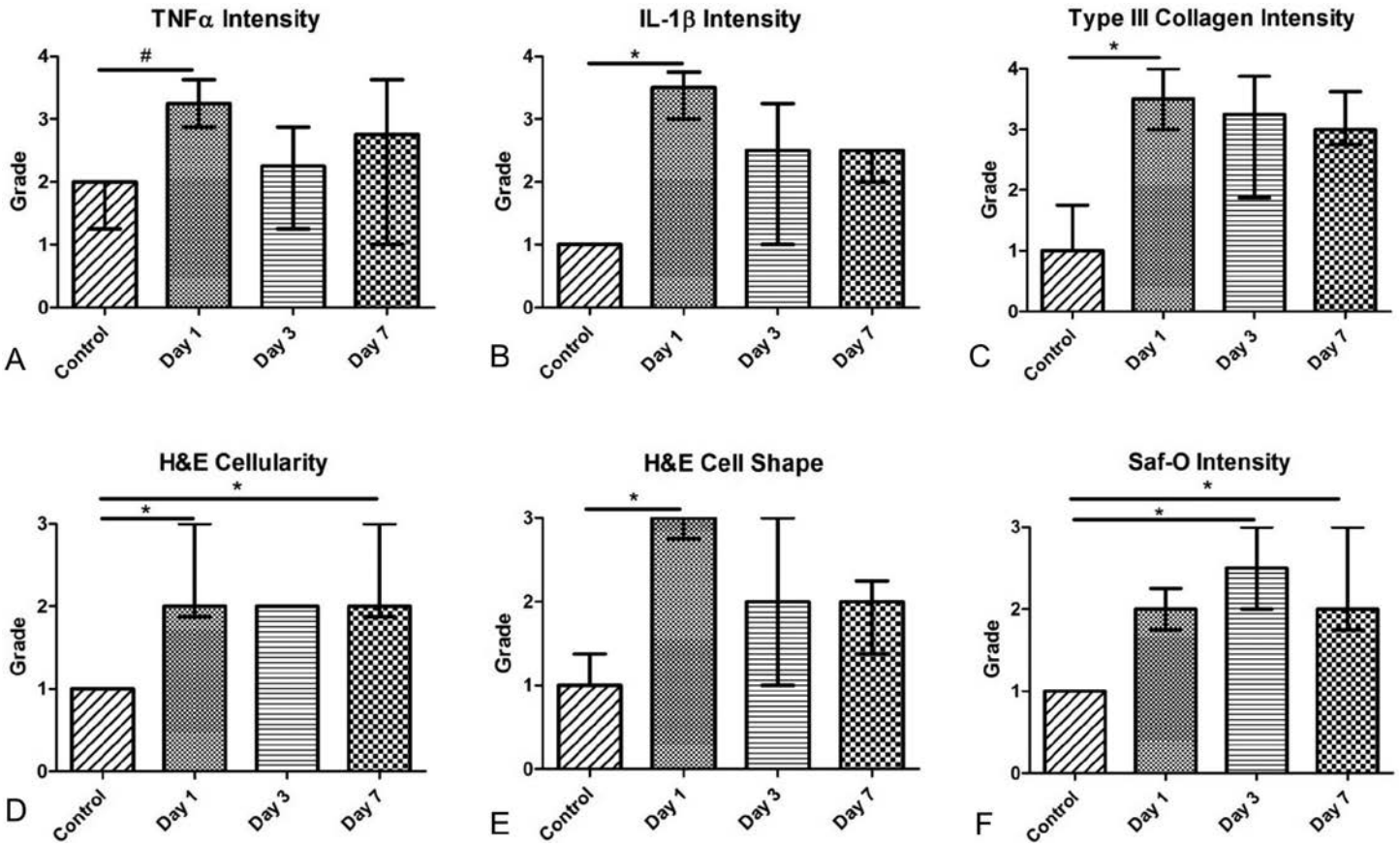

Figure 3. Histological evaluation the supraspinatus tendon 1, 3, and 7 days after the dry needling procedure compared to uninjured control. There was a trending increase in TNFa (A) at day 1, a significant increase in IL-1β (B), Type III Collagen (C), and rounded cell shape (E) at day 1, a significant increase in cellularity (D) at days 1 and 7, and a significant increase in GAG content (F) at days 3 and 7. Data presented as median and interquartile range.

the formation of granulation tissue in the damaged areas, which caused an increase in the cross sectional area and a subsequent decrease in material properties. Maximum load was not significantly different from the control, supporting that this injury may not dispose the tendon to early risk of failure. Further investigation is needed to more completely characterize the biologic response, as well as to evaluate later time points to determine the tendon healing potential to dry needling. This is the first study to demonstrate that the rat supraspinatus tendon can be dry needled consistently under ultrasound guidance, and that a controlled healing response can be elicited from this procedure. Further studies will apply this method in a tendinopathy model to evaluate the effect of dry needling on pathologic tissue.

Significance

Although dry needling is widely used in clinical practice for the treatment of tendinopathy, the lack of basic scientific studies examining the effects of this procedure is an important issue in preventing its recommendation for routine use. While further studies are necessary to examine the longer term effect on treated tendons, this study establishes a controlled model for evaluation of the mechanism and efficacy of dry needling in treating tendinopathy.

Acknowledgements

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