Aberrant Lymphatic Transport is Associated with Increased Interstitial Fluid Content after Achilles Tendon Injury

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Summary

Near-infrared (NIR) fluorescent tracers have been used to characterize lymphatic clearance pathways in healthy rat Achilles tendons. We applied these techniques in a tendon rupture model and found that while initial lymphatic clearance rates increase after injury, overall clearance slows significantly by 8 weeks. These data do not correlate directly to the time course of orthopedic functional recovery metrics, highlighting the complexity of the tendon healing process.

Introduction

Tendon's hypovascular nature is often blamed for its relatively poor healing capacity [1]. Tendon disease presents clinically with an array of symptoms and morphological changes, including an increase in interstitial fluid content that fails to resolve over time [2]. However, little is known about tendon microcirculation and fluid dynamics, specifically considering the role of the lymphatic system [3]. We showed in prior work that NIR tracers can be used to characterize microcirculatory transport in healthy rat Achilles tendons: small, vascular tracers cleared significantly faster than large, lymphatic tracers [4]. In this abstract we sought to understand the effect injury has on tendon lymphatic function, hypothesizing based on the recovery timeline of this injury model that lymphatic clearance would increase after 2 weeks, but would slow at the 4- and 8-week timepoints in line with injury resolution.

Methods

All procedures were approved by IACUC. Skeletally mature female Sprague Dawley rats were subjected to a unilateral full thickness Achilles tendon transection followed by suture repair. The injury model was characterized by *in vivo* functional tests (tactile allodynia and spontaneous weight bearing), *ex vivo* mechanical testing, and histology. At 1-, 3-, and 7-weeks after injury, contralateral and injured tendons received 5 µL co-injections of small and large NIR tracers. These tendons were imaged with an IVIS (Revvity) imaging system to quantify fluorescence over a week-long time frame. Normalized fluorescent data were fit with one phase exponential decay curves to quantify parameters related to clearance rates. Animals were euthanized (2, 4, and 8 weeks after injury) and their tendons were lyophilized to quantify the fluid content of the tissues. Data were analyzed via two-way ANOVA in Graphpad (Prism) with an alpha level of 0.05 and are presented as mean ± standard deviation.

Results and Discussion

Tendon injury significantly increased fluid content at all timepoints compared to baseline, peaking at 73.9% fluid mass at the 2-week timepoint (56.6% at baseline). This sustained increase in fluid content mimics the clinical presentation of tendon disease. Initial lymphatic clearance (Figure 1a) was significantly greater at 2 and 4 weeks compared to baseline and at all timepoints after injury compared to contralateral tendons. Overall clearance rates (Figure 1b) showed no change until 8 weeks after injury where lymphatic clearance was significantly slower than baseline. Over that same timeframe, weight bearing, tactile allodynia, and tensile testing displayed significant impairment to function that was eventually recovered either at 4 or 8 weeks. Representative histology demonstrated morphological improvements through the healing period but did not show a healthy phenotype by 8 weeks. When combined with lymphatic clearance data, these observations demonstrate that recovery cannot be described by just one parameter and emphasize the need for a more holistic view of functional recovery from injury.

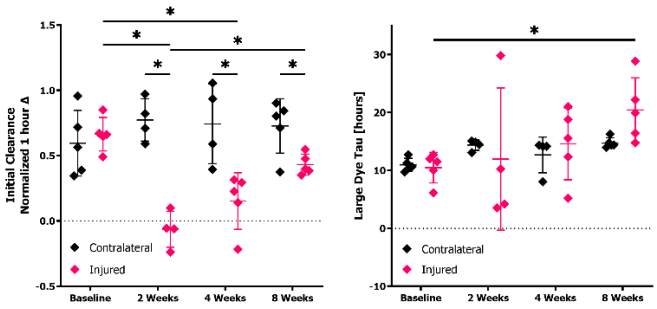

Figure 1: (a) Initial (first hour after injection) and (b) overall (time constant of decay) lymphatic clearance rates for contralateral and injured limbs at baseline and three timepoints after injury. Baseline values were captured from naïve animals. n = 4-5. *, $p<0.05$

Conclusions

Lymphatic microcirculation pathways in rat Achilles tendons are initially accelerated after injury but then slow with healing. Following work will perform immunostaining for endothelial cells of lymphatic capillaries in order to judge clearance rates in the context of lymphangiogenesis after injury.

Acknowledgments

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References

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