Achilles Tendon Repair Response to Injury is Enhanced by the Absence of Decorin

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Introduction

Achilles tendon ruptures are a common problem preventing athletic participation and daily life activities, often requiring surgical repair and extensive rehabilitation. In rabbit Achilles tenocytes *in vitro*, down-regulation of the proteoglycan decorin suppressed the expression of TGF-β1, a growth factor associated with fibrotic scar.[1] Similarly, in the medial collateral ligament *in vivo*, down-regulation of decorin improved healing.[2] Conversely, in mouse patellar tendon, the absence of decorin reduced uninjured tendon mechanical properties and had no effect on healing tendons 6 weeks after injury.[3] The effects of reduced decorin appear dependent on the site investigated[4] and its effect on Achilles tendon in particular is unknown. Since decorin plays a key role in collagen fibrillogenesis, inhibition of decorin would be expected to alter Achilles tendon properties. Therefore, the objective of this study was to investigate the effect of the absence of decorin *in vivo* on native and healing mouse Achilles tendons. We hypothesized that the absence of decorin would 1) impair the native mechanical properties of the Achilles tendon and 2) improve the healing mechanical properties of the Achilles tendon.

Methods

C57BL/6 wild type (WT) and decorin transgenic null (*Dcn−/−*) mice were used in this IACUC-approved study. Control, uninjured (UNJ) animals were sacrificed at 150 days of age (n=7-9 per genotype). Animals in the injured group (INJ) underwent a bilateral, centralized, full-thickness, partial-width injury to their Achilles tendon at 120 days of age (n=4-8 per genotype). For the injuries, animals were anesthetized, and skin incisions were made to visualize the Achilles tendons. Rubber-coated backings placed underneath the tendons provided support against a 0.5 mm biopsy punch used to create the defects. Incisions were sutured closed. Animals returned to cage activity and were sacrificed 3 weeks later.

At sacrifice, one Achilles tendon-calcaneus unit from each mouse was dissected. Tendon cross-sectional area was measured using a laser device. Stain dots were placed on the tendon for optical strain analysis. Tendons were stamped into a dog-bone shape to isolate the defect region. Cross-sectional area was also measured after stamping for use in calculation of material properties. The calcaneus and Achilles tendon were gripped in fixtures to create a gauge length of 5 mm from the bony insertion. The tendon was submerged in a 37°C heated PBS bath and tensile tested as follows: 1) preload, 2) preconditioning, 3-5) stress relaxation and sinusoidal frequency sweeps at 4%, 6%, and 8% strains, 6) return to gauge length, 7) ramp to failure at 0.1%/sec. The sinusoidal frequency sweeps were performed at 0.01, 1, and 10 Hz for 10 cycles at 0.125% strain for each frequency. The tensile modulus was determined from the ramp to failure. The dynamic modulus (|E*|, ratio of stress-to-strain amplitude) and tangent of the phase angle between the stress and strain (tan(δ), a measure of viscoelasticity) were calculated at each strain level and frequency.

Comparisons between genotypes for cross-sectional area and tensile modulus were made using separate t-tests for the UNJ and INJ groups. At each strain level, the effects of genotype and frequency on tan(δ) and |E*| were evaluated using a 2-way ANOVA, separately for the UNJ and INJ groups. When significant, p-values were calculated from t-tests comparing genotypes. Additionally, a bootstrapping technique, which creates random pairs of data to compare genotypes, was used to calculate the ratios of injured to uninjured data for tan(δ) and |E*| for each genotype.

Results

Dcn-/- uninjured tendons had increased area (Fig 1a), decreased linear modulus (Fig 1b), increased tan(δ) (Fig 2a), and decreased |E*| (Fig 2c) compared to WT. Frequency significantly affected tan(δ), but there were no interactions. Interestingly, results were opposite for injured tendons. *Dcn-/-* injured tendons had decreased area (Fig 1a), increased linear modulus (Fig 1b), decreased tan(δ) (Fig 2b), and increased |E*| (Fig 2d) compared to WT. For simplicity, results for tan(δ) and |E*| are shown for 8% strain; similar trends existed at the other strain levels. The bootstrapped data (not shown) confirmed that the ratio of INJ/UNJ resulted in cross-sectional area, linear modulus, tan(δ), and |E*| that were closer to native properties for the *Dcn-/-* group compared to WT.

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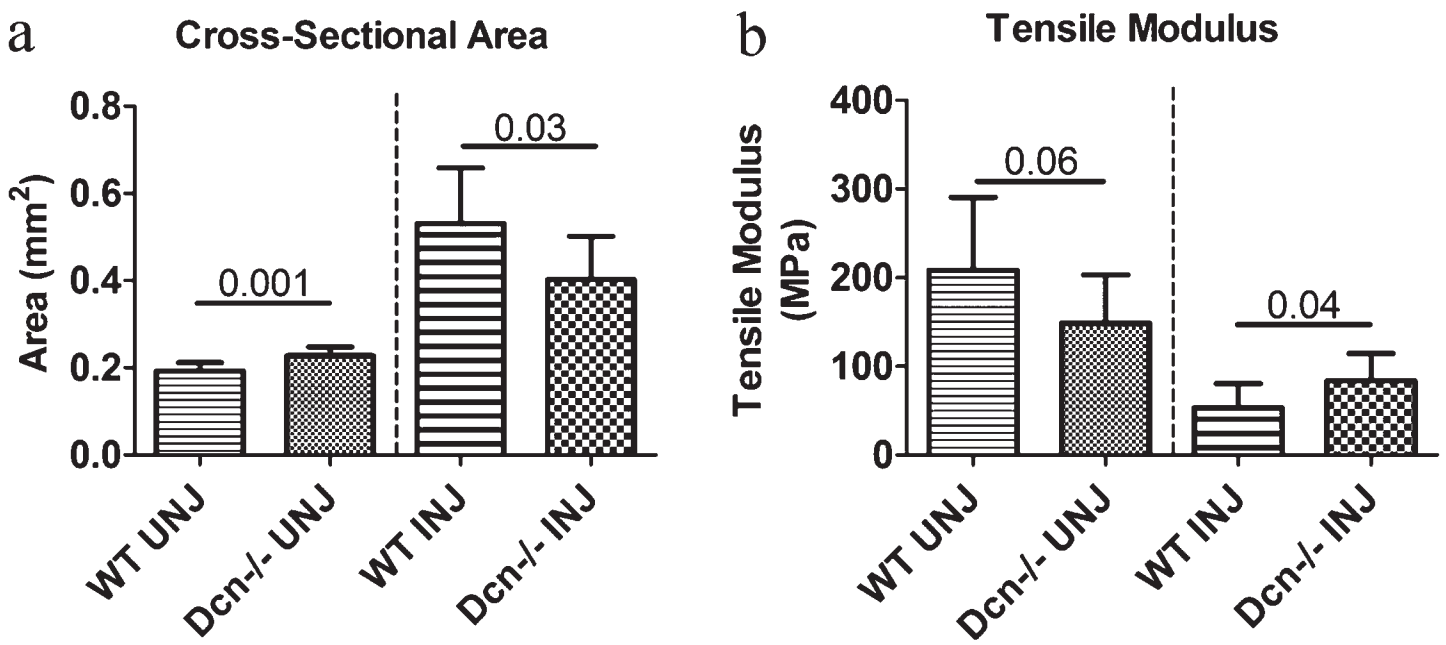

Figure 1. Uninjured *Dcn−/−* tendons (vs. WT) had increased cross-sectional area **(a)** and decreased tensile modulus **(b)**; injured *Dcn−/−* tendons (vs. WT) had decreased cross-sectional area **(a)** and increased tensile modulus **(b)**. Injured *Dcn−/−* tendons healed better than injured WT. mean = stdev.

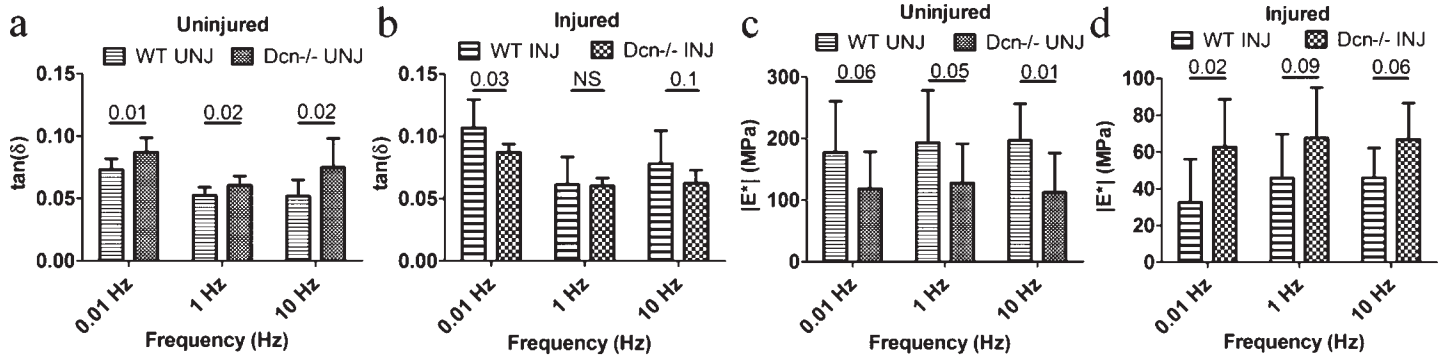

Figure 2. Uninjured *Dcn−/−* tendons (vs. WT) had increased tan(δ) **(a)** and decreased |E*| **(c)**; injured *Dcn−/−* tendons (vs. WT) had decreased tan(δ) **(b)** and increased |E*| **(d)**. Injured *Dcn−/−* tendons healed better than injured WT. Not shown are the differences between frequency for tan(δ): 0.01 Hz differed from both 1 Hz and 10 Hz ($p < 0.005$, Tukey's). mean = stdev, NS= not significant.

Discussion

Results support our hypotheses. Uninjured data suggests that decorin-deficient mouse Achilles tendons are mechanically weaker than wild type tendons. These results are in agreement with previous findings in the flexor digitorum longus tendon.[5] Injured *Dcn-/-* Achilles tendons healed better than WT tendons, as evidenced by the decreased area, increased tensile modulus, increased |E*|, and decreased tan(δ). The bootstrapped data further support that the decorin-deficient mice better recovered their native properties than the wild type mice 3 weeks after injury to their Achilles.

Decorin regulates the formation of larger collagen fibrils.[6] Inhibition of decorin during tendon healing may reduce scarring and improve collagen fibrillogenesis by allowing the formation of more mechanically stable collagen fibrils. Future work includes testing biglycan transgenic null tendons, examining a later time point, and performing additional assays to elucidate the mechanisms responsible for the differences between genotypes and the function of decorin in these processes.

Significance

The absence of decorin impairs native mouse Achilles tendon mechanical properties but enhances healing mechanical properties at a 3 week time point. This study provides support for reducing decorin expression as a treatment to improve Achilles tendon healing.

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