Injured Tendons Increase Lactate Synthesis and Pharmacological Inhibition of Lactate Synthesis Improves Tendon Repair.

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Introduction

Incomplete tendon healing leads to significant mobility restriction, pain and substantial health care costs. To develop novel targeted therapies for tendon injury, it is necessary to define the molecular changes and mechanisms governing the tendon healing process. Up-regulation of glycolysis and lactate synthesis occurs in wound, inflammation and cancer. Recently, we have found that IL-1 inhibits tenogenic differentiation of injured-tendon derived progenitors and increases their lactate synthesis and that inhibition of lactate synthesis competed against the IL-1 action on tenogenic differentiation.[1] We also know that inflammatory cytokines are up-regulated in injured tendons.[2] Taken together, we hypothesize that tendons increases lactate synthesis in response to injury and pharmacological inhibition of this alteration is beneficial for tendon repair. We analyzed activities of glycolysis and lactate synthesis in injured tendons with ^{13}C-glucose labeling and examined the effects of dichloroacetat (DCA), an inhibitor of lactate synthesis, on recovery of collagen fiber formation and biomechanical properties in the mouse achilles tendon injury model.

Methods

All animal experiment procedures were approved by the Institutional Animal Care and Use Committee of the Children's Hospital of Philadelphia. *Tendon surgery:* A complete transverse incision was made at the midpoint of the right Achilles tendon in 8-week-old female C57/BL6 mice and the gap was left open.[3] Animals were returned to cage activity and euthanized 1 or 4 weeks after surgery, representing the inflammation/proliferation and repair phases, respectively. Harvested tendons were subjected to metabolomics, histological and biomechanical analyses. *^{13}C-glucose labeling*: 13C-glucose (400mg/kg) was peritoneally injected 1 h prior to euthanization. The uninjured or injured tendons ($n = 4$) were snap-frozen in liquid nitrogen and subsequently ground to powder for perchloric acid extraction. ^{13}C-metabolites and intermediates were analyzed by combination of LC-MS and MC-MS.[4] *DCA administration:* Dichloroacetate (DCA) (100 mg/kg, daily) was given to C57/BL6 mice from 1 day to 4 weeks post-surgery. *Mechanical testing:* Achilles tendons with attached calcanei ($n = 10$) were fine dissected and hydrated in PBS. Tendon cross-sectional areas were calculated with a custom laser-based device.[5] For tensile testing to failure, tendons were placed in a custom fixture that grips the calcaneus and tendon ends. Fiber alignment measures were collected during mechanical testing with a cross-polarizing technique.[5] *Statistics:* Student's t-tests or two-way factorial ANOVA followed by Boneferroni post-hoc multiple comparison tests were used to identify differences between groups. Significance for all tests was set as $p < 0.05$.

Results

The molar percent enrichment of ^{13}C-latate was strongly increased at 1 week post-injury and remained high after 4 weeks (Figure 1). In addition, enrichment of ^{13}C-glyceraldehyde, a metabolite in glycolysis pathway, was significantly higher in injured tendons in the 1week group compared to uninjured tendons. Four weeks after injury, ^{13}C-glyceraldehyde enrichment decreased, but was still higher than the uninjured tendon (Figure 1). DCA-treated samples had smaller cross sectional areas (Figure 2A Analysis of axial view of collagen fibers by electron microscopy revealed that the DCA-treated tendon contained thicker fibers. Finally biomechanical assessments demonstrated that modulus and maximum strength were significantly higher in the DCA-treated tendons than the vehicle-treated tendons (Figure 2B and C). In addition, the DCA-treated tendons had a lower circular variance, which is indicative of better alignment (Figure 2D).

Discussion

The results indicate that injured tendon acutely increases glycolysis and lactate synthesis and that inhibition of lactate synthesis improves recovery of collagen fiber structure and biomechanical properties. Alterations of glucose metabolism were found not only in an inflammation phase but also in the repair phase, indicating that the responsible cells for the alterations are not only in inflammatory cells and vessels but also the tendon cells and tendon progenitors that contribute to tendon regeneration. Thus, the findings indicate that

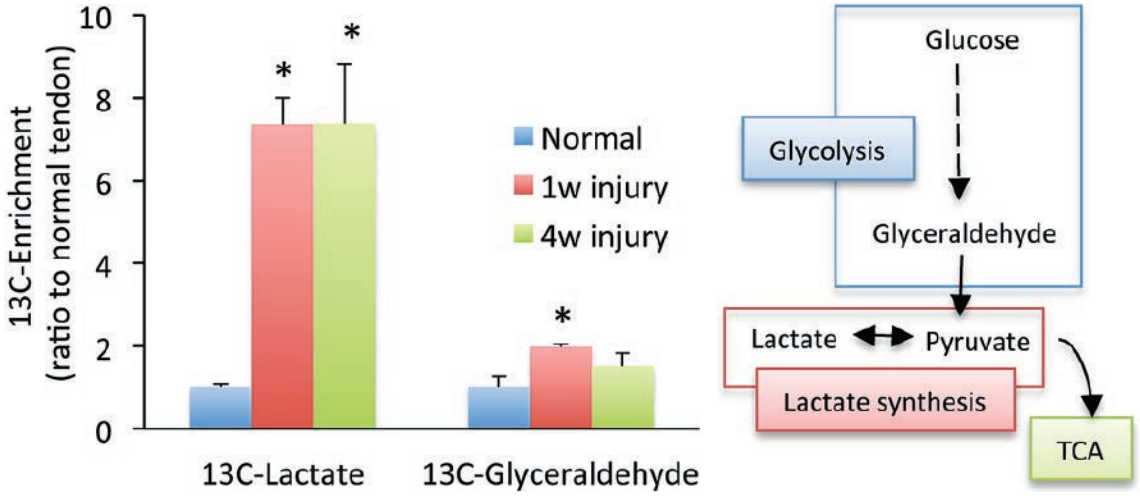

Figure 1. Injured tendons increased an influx of glucose to glycolysis and lactate synthesis pathway. 13C-glucose was injected 1 h in prior to euthanization. The uninjured or injured tendons (1 or 4 weeks postinjury) were harvested and subjected to metaboromics analysis to measure the molar percent enrichment of 13C-metabolites. *, $p < 0.05$.

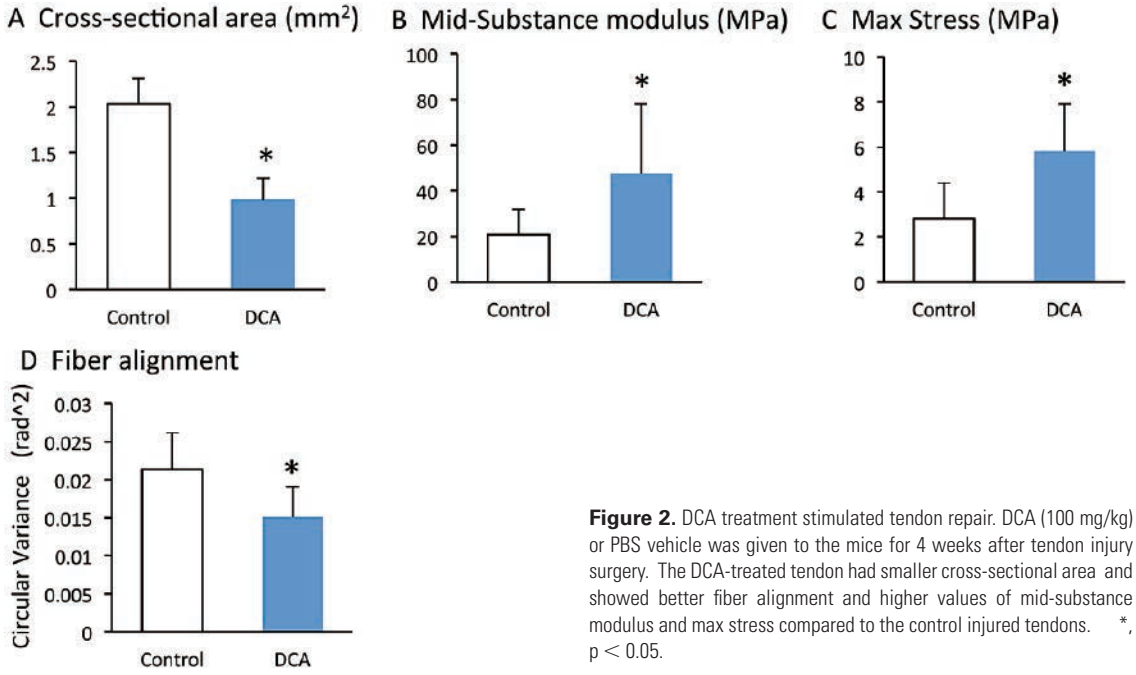

Figure 2. DCA treatment stimulated tendon repair. DCA (100 mg/kg) or PBS vehicle was given to the mice for 4 weeks after tendon injury surgery. The DCA-treated tendon had smaller cross-sectional area and showed better fiber alignment and higher values of mid-substance modulus and max stress compared to the control injured tendons. *, $p < 0.05$.

injured tendons reprogram glucose metabolism and that metabolic drugs can modify this alteration and improve tendon healing. In wounds, lactate accumulates regardless of oxygen concentration and stimulates VEGF in macrophages and collagen synthesis in fibroblasts.[6] Lactate my mediate angiogenesis and fibrous tissue formation (scar) in injured tendons.

Significance

While a large number of clinical and preclinical approaches have been attempted, none result in complete recovery of mechanical structure and function in injured tendons. This study provides direct evidence that glycolysis and lactate synthesis can be novel therapeutic targets for tendon repair.

Acknowledgments

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