Microdialysis as a Longitudinal, In Vivo Assessment of Achilles Tendon Healing in a Rat Model

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Introduction

The Achilles tendon is the most frequently ruptured tendon, leading to significant pain, loss of function, and healthcare costs.[1] In vivo assessment of healing after an Achilles tendon rupture can provide valuable metrics not only to monitor healing, but also to guide treatment options.[2,3] Specifically, in vivo assays such as ultrasound imaging, passive joint mobility assessments, and functional gait analysis can provide longitudinal measures of structural and functional properties of the healing tendon. However, these assays do not provide insight into the biologic changes in the healing tendon.[4-6] While microdialysis has been used to assess tendon healing in humans, it has not been used in an animal model of Achilles tendon injury.[7,8] Therefore, the objective of this study was to develop and pilot a novel use of microdialysis in vivo to directly measure key biologic markers of tendon healing and matrix deposition in the rat Achilles tendon. We hypothesized that, following Achilles injury, metabolite and procollagen concentrations would significantly increaseindicating higher metabolic activity and collagen synthesis, respectively.

Methods

Experimental Design

After facility acclimation, six, 4-month male Sprague Dawley rats underwent unilateral blunt transection of the right Achilles tendon without repair (IACUC approved). The right hind limb was immobilized for 7 days. Microdialysis measurements were taken before injury and 7, 14, and 21 days post injury.

Dialysate Collection and Analysis

Under isofluorane anesthesia and ultrasound guidance, a microdialysis catheter (CMA 71; CMA Microdialysis AB; 100kDa molecular cutoff, 0.5mm outer diameter; 4mm in length) was introduced from the proximal aspect of the tendon towards the calcaneus. The active part of the membrane was placed in the rupture site and a perfusion fluid of artificial CSF with 3% 500kDa dextran (Sigma Aldrich) was used. The fluid was pumped through the inner tube of the catheter into the space between the inner tube and the semipermeable catheter membrane, where the exchange between the interstitial and perfusion fluid takes place. The resultant dialysate solution was transmitted from the catheter and collected in a 1.5mL vial (Microvial, CMA Microanalysis AV). With a perfusion speed of 1.0 μL/min, samples were collected for 2.5 hours. Due to fluid pump adjustment during the first few minutes, trauma from the probe insertion, and to remain conservative, the first 30 minutes of dialysate was discarded. Lactate, pyruvate, glucose, glutamate, glycerol, and procollagen type I N propeptide (PINP), concentrations were quantified via ELISAs.

Statistics

All comparisons were made using the nonparametric Kruskal-Wallis ANOVA followed by Dunn's post hoc tests, which compared values at 7, 14, and 21 days post injury to preinjury values.

Results

Lactate (Fig.1A) and pyruvate (Fig.1B) concentrations significantly increased 7 days post-injury, with no changes in lactate:pyruvate ratio at any time points (Fig.1C). Glucose concentration 7 days post-injury showed significant increases (Fig.1D). Glutamate was elevated 21 days following injury (Fig.1E). No changes were found in glycerol concentration following injury (Fig.1F). PINP concentrations were decreased at each post-injury time point compared to pre-injury measures (Fig.2).

Discussion

Results indicate an early increase in overall metabolic activity and simultaneous decrease in collagen I production following Achilles injury. Increases in lactate and pyruvate 7 days post-injury indicate increased anaerobic and aerobic metabolic activity, respectively, as the resident cell population begins tissue repair. No changes in the lactate:pyruvate ratio demonstrate that the local environment is sufficiently oxygenated, as aerobic and anaerobic activity levels are maintained throughout healing.[7] Under normal healing conditions, angiogenesis peaks around day 7 in a healing tendon,[9] which is supported by the early increase in glucose concentration. This increase in glucose concentration may also indicate increased metabolic activity immediately following injury, concurrent with the lactate and

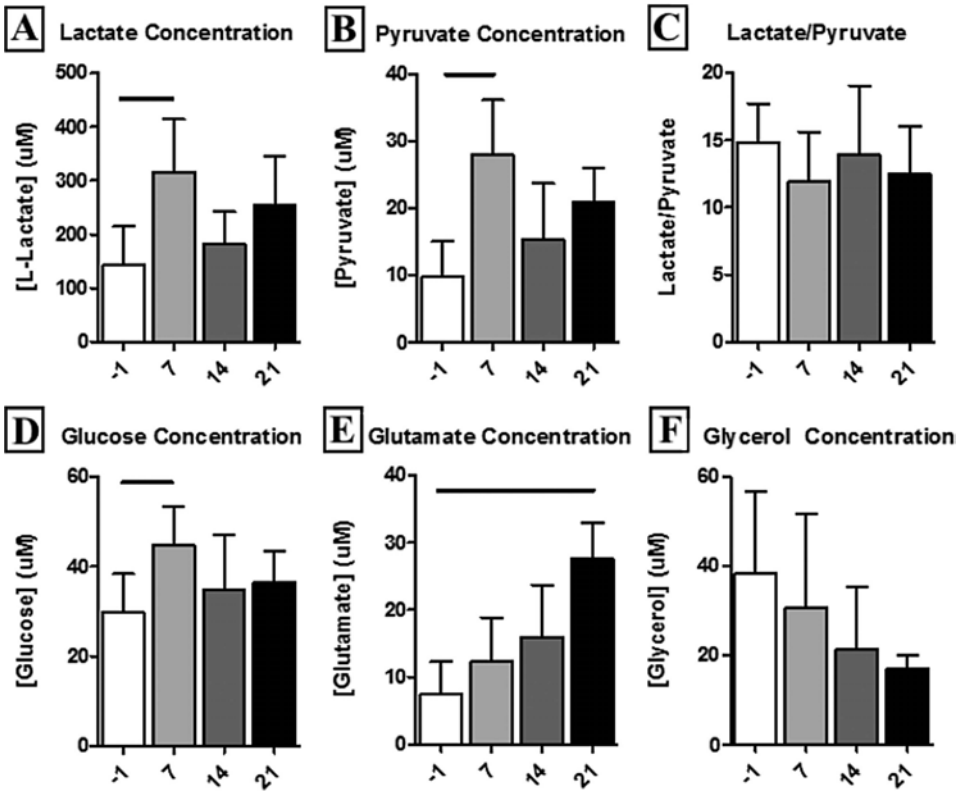

Figure 1. ELISA results from dialysate prior to injury (−1) and 7, 14, and 21 days post injury. Lactate **(A)** and pyruvate **(B)** concentrations peaked at 7 days postinjury while their relative ratio **(C)** was maintained. Glucose **(D)** peaked at day 7. Glutamate (E) was significantly increased at day 21. No changes were found in glycerol **(F)**. Data as mean +/− standard deviations; bar indicates significance.

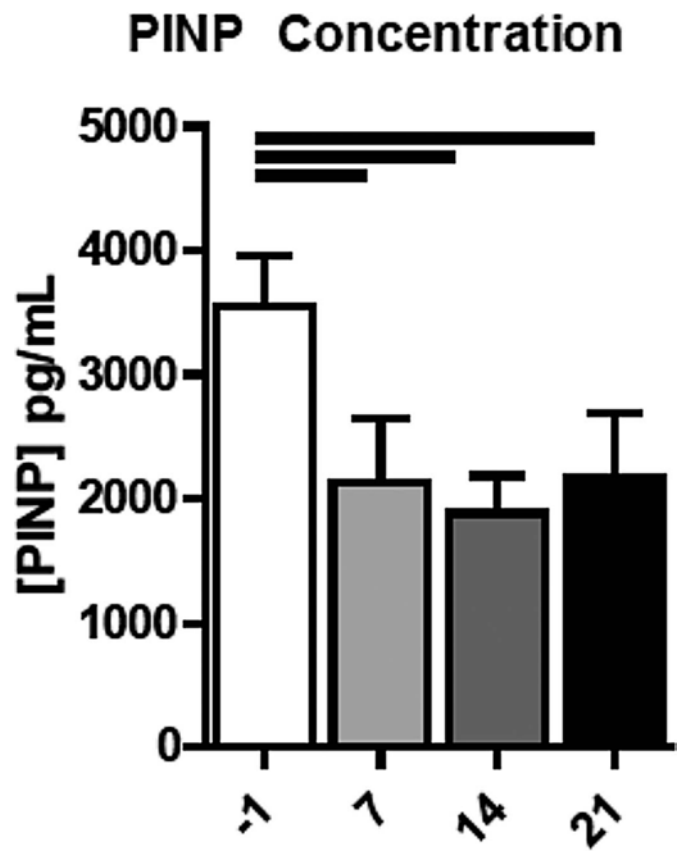

Figure 2. ELISA results showed a decrease in PINP concentration throughout healing compared to pre-injury levels. Data as mean +/− standard deviations; bar indicates significance.

pyruvate changes shown. Glutamate concentration peaks at 21 days post-injury in congruence with nerve ingrowth.[7] Glycerol is a marker for cellular damage, and results show no changes in the metabolite's concentration, thus the severity of cellular damage remains unclear in our injury model.[7,8] PINP decreased immediately following injury, demonstrating a reduction in collagen I production. The study timeline was likely not long enough to see the expected increase in collagen I production as the tendon begins the remodeling phase of healing in which collagen III in the fibrotic scar tissue is replaced by more aligned collagen I.[2] Future studies will investigate changes in the biological environment of a healing Achilles tendon in response to exercise and new modalities to improve healing outcomes.

Significance

This study demonstrates that microdialysis is a viable in vivo, longitudinal measure of Achilles tendon healing in a rat model. This technique will provide valuable metrics to monitor the biological environment in healing Achilles tendons.

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