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Original Article

Improved Achilles tendon healing by early mechanical loading in a rabbit model

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Abstract: Objective: To investigate the structure and the attachment strength of a healing tendon-bone interface and the role of mechanical loading in tendon healing. Methods: Sixty rabbits underwent unilateral detachment and repair of the Achilles tendon. Thirty animals were immobilized (Group A), and the others wereallowed loadingimmediately postoperatively (Group B). Animals were sacrificed at 4 weeks and evaluated for histological and biomechanical testing. Statistical analysis was performed with an independent t test with significance set at $P = 0.05$. Results: The ultimate stress was greater in group B (4.598 ± 1.321 N/mm^2) compared with the control group (3.388 ± 0.994 N/mm^2) ($P < 0.05$). Similarly, a more organized tendon-to-bone interface with a larger area of chondrocytes was found in group B ($P < 0.05$). Conclusion: Mechanical loading improves the structure and the attachment strength of the healing tendon-to-bone interface.

Keywords: Achilles tendon healing, mechanical loading, rabbit model

Introduction

The healing of tendon to bone is a basic requirement for the long-term survival of tendon surgery. The weakest link following reconstruction is not the graft itself but the fixation between the graft and the bone until graft osseointegration occurs [1, 2]. The tendon-bone insertion is a structure that links tendon and bone-two materials that exhibit dramatically different mechanical behaviors. The insertion site consists of a gradation of cell types, collagen types, mineral contents and collagen fiber orientations. However, this complex attachment creates a particularly difficult challenge in effectively responding to injuries and achieving reconstruction following surgery.Repair site healing is characterized by poor-quality scarring that remains of poor quality long after surgery [3].

The healing process of the tendon-to-bone interface isinfluenced by a number of environmental, biological, andmechanical factors. In particular, the role of the mechanicalenvironment, as influenced by postoperative rehabilitation,significantly affects tendon-to-bone healing.Previous studies have demonstrated that mechanical loading plays a critical role in maintaining the homeostasis of native musculoskeletal tissue [4, 5]. Additionally, increased force is beneficial to healing in a number ofmusculoskeletal tissues; for example, increased compressionimproves bone fracture healing, and early mobilizationimproves anterior cruciate ligament healing [6, 7]. Uninjured ligaments and tendons as well as their entheses are sensitive to their mechanical environments and generally demonstrate increases in the tensile modulus in response to increasing loads [4]. In contrast, stress deprivation is associated with a decline in mechanical properties [5, 8, 9]. The influence of mechanical loads on a healing enthesis, however, remains unclear, and studies have been limited by an inability to quantify or control the loads applied to the tendon-bone interface.

We developed a rabbit model of Achilles tendon reconstruction that allowed for controlled cyclic axial loading of the healing tendon-bone interface. The purpose of this study was to determine the effect of mechanical load on healing between soft tissue and bone. Our overall hypothesis was that loading would improve tendon-to-bone healing compared with that resulting from prolonged immobilization.

Method

This study was approved by our Institutional Review Board and Institutional Animal Care and Use Committee. A total of 60rabbits (Shanghai Super, B&K Laboratory Animal Corp. Ltd.) were used in thisstudy.The rabbits were randomly assigned to one of two postoperative regimens: postoperative immobilization in a cast for 4 weeks (Group A, n = 30) or immediate postoperative loading (Group B, n = 30). The animals were euthanized at 28 days (4 weeks) postoperatively for biomechanical testing and histomorphometric analysis of the bone-tendon-bone complex.

Animal surgery

The animals wereanesthetized with an intravenous injection of Nembutal (pentobarbital, 50 mg/kg). The hind limbs were shavedand prepped with alternating alcohol and chlorhexidinescrubs in triplicate. A longitudinal posterior midline incision and medial andlateral full-thickness skin flaps were made to identify and isolate the Achilles tendon. The Achilles tend on was detached from its footprint. A #15 blade knife was used to clear the fibrocartilage completely of the calcaneus, leaving a bleeding bone. Next, the Achilles tendon ends were attached to the calcaneus, using the appropriate suture repair type, with a figure-of-eight stitch. The wound was irrigated and closed with 4-0 monofilament suture.

Histomorphometric analysis

Ten animals in each group were euthanized to obtain specimens consisting only of the calcaneus with the attachedAchilles tendon and muscle at 4 weeks for histologic examination. The specimens were fixed in 10% neutral buffered formalin, then decalcified with Immunocal, and finally embedded in paraffin with the tendon at approximately 45° from the bone. Multiple sections of 5 microns thick were cut in the coronal plane through the repairedAchilles tendonand the insertion into the calcaneus. Then, the sections were stained with hematoxylin and eosin (H&E) and safraninO/fast green. A light microscope was used to examine the calcaneal tuberosity specimens, the repaired tendon-bone insertionsites, and the mid-substances of the Achilles tendons. Digital images were taken using a SPOT RT camera, and semi-quantitative analyses were performed using computerized image analysis to determine the amount of new fibrocartilage based on the areas of metachromasia with safraninO staining. SafraninO/fast-green stains proteoglycans reddish-purple, a phenomenonreferred to as metachromasia. ImageJ software was usedto manually outline the areas of metachromasia on safraninO slides at 40 × magnification on 4 sequential coronal sections.The total area for each specimen was then recordedas micrometers squared, and the mean plus or minus standarddeviation for each group was determined [10]. Semi-quantitative histomorphometric analyses were performed by 3 independent observers who were blinded to the study.

Biomechanical testing

At week 4, 20 animals per group were euthanized for biomechanical testing. The Achilles tendon was isolated carefully to ensure that the muscle belly and the sutures were detected away from the tendon. The specimens were wrapped in gauze soaked in phosphate-buffferedsaline (Gibco) and stored at -80°C until the time of analysis. At the time of testing, the specimens were thawed at roomtemperature overnight, and all excess muscleand the sutures were carefully removed. Digital calipers were used to measure the cross-sectional area of theAchilles-tendon-to-boneinterface. The specimen was then transferred to a uniaxial testing system. The tendon was secured in a screw grip using sandpaperand ethyl cyanoacrylate glue. The calcaneus was secured ina custom-designed vise grip. The tendon was securedto a 45-N load cell attached to a linear bearing that allowedalignment of the tendon in the direction of its pull. The tendon was preloaded to 0.10 N and then loaded to failure at a rate of 14 microns/s until the tendon-to-bone interface failed, at which point the maximum loads at failure were recorded and a 1-micron resolution micrometer was used to measure the displacement. The ultimate stress index (USI) at failure was defined by dividing the ultimate force at failure by the cross-sectional area.

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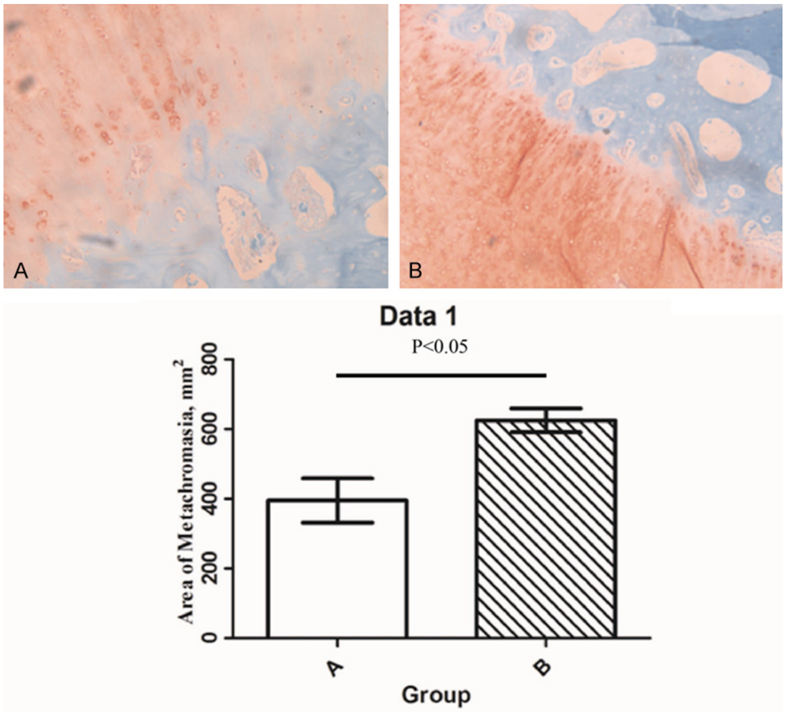

Figure 1. The comparison of the fibrocartilage between the groups with independent sample t tests ($P < 0.05$). Representative histology images of cartilage at theinsertionsites. Slides were prepared with safranin0/fast greenstains that stained the proteoglycans in cartilage a magenta colour. There was a greater area of metachromasia found in the loading group compared with the immobilizationgroup at 4 weeks.

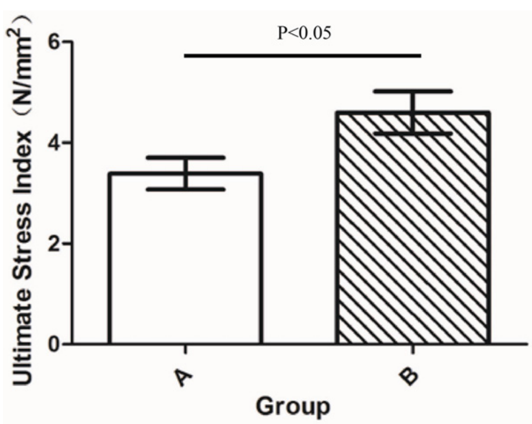

Figure 2. The comparison of the ultimate stress indices between the groups with independent sample t tests ($P < 0.05$). There were higher ultimate stress indices in the loading group comparedwith the immobilizationgroup at 4 weeks.

Statistical analysis

The primary outcome of this study was establishingthe biomechanical integrity of the tendon-to-bone attachment. A power analysis (PASS 11) was performed with the data from our preliminary experiment that investigated the ultimate stress of the tendon-to-bone healing. Using these estimations, a power of 0.80 was achieved using at least 20 specimens per group with a = 0.05 for biomechanical testing to identify a significant difference with a Type I error of .05. An additional 10 tendons per treatment group per time period were required for histologic evaluation. The statistical analysis was performed using SPSS software (version 13.0). The data between the groups were compared with independent sample t tests. A difference with a *P* value of less than 0.05 (5%) was interpreted as significant. Outcomes were reported as the mean ± S.D.

Results

Thetendon-to-bone interface had healed in all animals at the time of necropsy. There were nosigns of infection in any specimen. There were no appreciable differences between the groups.

Histomorphometric analysis

There was a poorly organized, highly cellular, fibrovascular granulation tissue at the tendon-to-bone interface in group B. But in group A, the interface was less cellular and began to show matrix organization in line with the tensile pull of the tendon. Fibrocartilage was seen starting to form at the interface; however, the chondrocytes were immature and disorganized in group B. And there was more fibrocartilage, and this fibrocartilage was more organized in group A. Fibrovascular scar tissue was found at the tendon-bone interface in all specimens. There was markedly more fibrocartilage ($P < 0.05$) at the tendon-bone interface in group B (625.3 ± 109.5 mm^2) compared with Group A (395.3 ± 201.3 mm^2) (**Figure 1**).

Biomechanical testing

Similar results were found with regard to the biomechanical testing. At 4 weeks, the ultimate stress index was significantly greater ($P < 0.05$)

in group B (4.598 ± 1.321 N/mm^2) than in group A (3.388 ± 0.994 N/mm^2) (**Figure 2**).

Discussion

The purpose of this study was to determine the effect of mechanical loading on tendon-to-bone healing after Achilles tendon reconstruction. We hypothesized that mechanical stimulation would provide an improved healing response compared with that resulting from prolonged immobilization. The results demonstrated significant improvement in the structure and the attachment strength of the healing tendon-to-bone interface in the group with mechanical loading.

It is known that loading and tension play a large role in overall musculoskeletal tissue function. Musculoskeletal tissues respond to stress, both in normal homeostatic and in injured or healing conditions. The factors that affect healing are partly environmental and biological, but the mechanical environment also has significant influence. Previous studies have clearly demonstrated that mechanical loading plays a critical role in maintaining the homeostasis of both the biological response and the mechanical behavior of native musculoskeletal tissue. Uninjured ligaments and tendons as well as their entheses are sensitive to their mechanical environments and generally demonstrate an increase in tensile modulus in response to increasing loads, and stress deprivation is correspondingly associated with a decline in mechanical properties [5, 8, 11-13]. Similarly, bone is very sensitive to mechanical loads. Increased loading promotes new-bone formation and increased mechanical properties, whereas stress deprivation can lead to profound decreases in bone mineral density and rapid resorption.

After tendon injury, operative intervention is often necessary to restore function. Previous work has shown inferior healing when operative repair is delayed following injury [3, 14]. Although current therapies produce functional outcomes in the short term, long-term repair outcomes vary with respect to type of injury, injury location, and severity [15]. A large number of tendon injuries result from ruptures at the tendon-to-bone insertion site. The tendon-bone insertion is a structure that links tendon and bone-two materials that exhibit dramatically different mechanical behaviors. This complex zonal interface is characterized by the integration of a tendon's collagen fibers transitioning through a fibro cartilaginous region into the mineralized bone. The differences in the material properties of the soft and hard tissue lead to high stress concentrations at this site, contributing to injury [16-19]. As a result, it is particularly difficult to effectively respond to these injuries and achieve reconstruction following surgery. In an effort to improve tendon-to-bone healing, the application of static or cyclic loading at the insertion site may be necessary to restore the zonal phenotype [16, 20, 21]. Additionally, the repair tension, the amount of tension placed on a tendon to reattach it to the bone, is important in recreating theinsertion site [3].

The influence of mechanical loads on a healing en thesis has been controversial. For example, in a model of flexor tendon injury, the muscle loading was as beneficial to healing at the tendon-bone interface as it was to the biomechanical properties [13]. However, a prior study by the same group found that early exercise after repair of an acute rotator cuff tear impaired healing compared with that resulting from immobilization [8]. Sakai et al. similarly found healing and graft attachment strength to be better with postoperative immobilization [9].

Clinical studies have also been performed to address the role of mechanical stimulation. Ito et al. found no significant differences in anterior laxity, joint proprioception, or thigh muscle strength at one year postoperatively between the groups with and without mechanical loading [22]. Henriksson et al. randomized patients to be managed with an early range of motion or with plaster cast immobilization, and they found no significant differences [23]. Gomez et al. showed that increased static stress was beneficial to the properties of the healing medial collateral ligament [24]. Increased cyclic stress, in contrast, was detrimental to medial collateral ligament healing [11].

Our observations of the improved biomechanical and histological properties of the healing en thesis after Achilles tendon reconstruction when mechanical loads were applied supported our main hypothesis.

Conclusion

In conclusion, we found that mechanical stimulation was beneficial to tendon-to-bone healing in a rabbit model.

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Disclosure of conflict of interest

None.

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