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ORIGINAL RESEARCH ARTICLE

Contrast-enhanced Sonography for the Evaluation of Neovascularization in Tendinopathic Tissues

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Objective: The purpose of this study was to evaluate the usefulness of contrast-enhanced ultrasound for detecting neovascularization in tendinopathic tissues using an animal model.
Methods: Doses of 100 μL and 50 μL collagenase were injected into the left and right Achilles tendons of six rabbits. Power Doppler ultrasonography was used before and after the administration of a contrast agent to evaluate tendon perfusion on Day 0 and Day 14 after the collagenase injections. The number of color pixels within the region of interest represented the amount of vascularity.
Results: The tendon cross-sectional area was similar between Day 0 and Day 14, and comparisons between both tendons also appeared to be insignificantly different between these two times. Noncontrast power Doppler ultrasonography failed to detect more color pixels in the tendons after collagenase injections in comparison with baseline, whereas a higher peak signal intensity was identified using contrast enhancement in the collagenase-treated tendons. In addition, differences in vascularity between the tendons that received different amounts of collagenase were clearly revealed after the administration of the contrast agents.
Conclusion: The present study demonstrates that contrast-enhanced ultrasound is superior to the noncontrast ultrasound for measuring hypervascularity in Achilles tendinopathy in a rabbit model.

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Introduction

The Achilles tendon, the largest and strongest tendon in the human body, is derived from the soleus and gastrocnemius muscles and inserts posteriorly to the calcaneus. Achilles tendinopathy commonly occurs in competitive and recreational athletes [1]. Observational data suggest that competitive athletes have a lifetime incidence of Achilles tendinopathy of up to 24% [2]. The etiology of Achilles tendinopathy is multifactorial. Intrinsic factors include impaired tendon perfusion, older age, female sex, heavy weight, taller heel height, and ankle instability [3], whereas extrinsic causes include previous lower limb injury and vigorous physical activities [4]. The Achilles tendon has a hypovascular zone of 4—6 cm caudal to its calcaneal insertion site, where tendon degeneration and tears commonly occur [5, 6]. In the histology of Achilles tendinopathy, interfibrillar glycosaminoglycan proliferation and collagen fiber disarrangement commonly present, but not with inflammatory cell infiltration [1]. Some previous studies have suggested that neovascularization contributes to painful Achilles tendinopathy. Previous studies have described sclerosing neovessels in order to relieve pain in most patients [7]. One study found that higher levels of glutamate receptors and vascular endothelial growth factors exist in the vicinity of the nerves in patients with painful Achilles tendinopathy, suggesting that the increase in nociceptive nerve fibers and neovascularization occurs in Achilles tendinopathy [8].

Several imaging tools have been used to evaluate Achilles tendinopathy, including plain film, computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound. Plain film is inexpensive and can be used to screen for the existence of bony abnormalities. The CT scan demonstrates a higher sensitivity for detecting calcifications and is useful for planning surgeries to treat complicated foot and ankle fractures. However, both imaging modalities fail to delineate tendon morphology and also result in increased radiation exposure. MRI depicts the multiplanar anatomy at a high resolution, but it is sensitive to changes in water content in tendon tissues. Ultrasound, in comparison with MRI, is less costly, provides an easy dynamic evaluation, and allows real-time comparisons with painful sites. Therapeutic injection can also be administered under ultrasonographic guidance [5]. Grey-scale sonography has also been used to reveal that normal Achilles tendons are composed of parallel arrays of fibrillar lines in longitudinal planes and appear round-to-ovoid along transverse planes. Focal hypoechogenic changes, such as the thickening of the Achilles tendon, are considered pathological findings. Because neovascularization is often associated with painful Achilles tendinopathy, we can use color or power Doppler to detect blood flow in a tendon. A previous study demonstrated neovascularization in all symptomatic tendons [9]. Power Doppler is superior to color Doppler because of its sensitivity to blood flow, and it is relatively independent of the angle of incidence. Nevertheless, power Doppler is still inadequate for recognizing the vascularity of hypovascular structures, such as tendons [7,10]. Because neovascularization is highly correlated to painful Achilles tendinopathy, this study sought to determine whether contrast-enhanced ultrasound is more effective than traditional power Doppler for investigating hypervascularity in Achilles tendinopathy.

Materials and methods

Animal model

We examined six male New Zealand rabbits (mean weight: 3.2 kg; range: 3.1—3.4 kg) and both Achilles tendons of each rabbit. All of the rabbits were anesthetized with isoflurane, and the hairs of the hind feet were shaved. According to our pilot study, a subcutaneous injection of 100 μL (10 mg/mL) collagenase (Sigma-Aldrich, St. Louis, MO, USA) is adequate for inducing swelling and local erythematous changes over the Achilles tendons, and the gross appearance typically returns to baseline approximately 2 weeks later. We injected 100 μL and 50 μL of collagenase into the left and right leg of each rabbit, respectively, at 1.5 cm proximal to the bony insertions of the Achilles tendons. We performed the ultrasonographic measurement on Day 0 before collagenase injection and on Day 14 after injection. This project was approved by the animal ethics committee of National Taiwan University Hospital.

Ultrasound imaging and protocol

We used an ultrasound machine (Acuson S2000 system, Siemens, Munich, Germany) equipped with a multifrequency transducer (14L5, 6—12 MHz in 2D mode; 5.5—7.5 MHz in Doppler mode) to perform the examinations. Power Doppler was employed to investigate tendon vascularity and was adjusted to the highest sensitivity that did not result in artifacts beneath the bony cortex before administering contrast agents. In order to reduce the blooming effect, the color gain was reduced to 0 dB while contrast agents were being introduced. A video retriever was externally connected to the ultrasound machine for continuous recording. The ultrasound examinations were performed on Day 0 before collagenase injection and on Day 14 after injection. The rabbits were settled in the prone position with the lower limbs immersed in a water tank, and the ultrasound probe was fixed along the long axis of the Achilles tendons (Fig. 1). Grey-color imaging was used to locate the midpoint of the calcaneus bone in the transverse plane, and then the probe was turned parallel to the long axis of the Achilles tendons. The right vertical border of the scan view was adjusted to pass through the caudal edge of the calcaneus bone in order to assure that the same segment was investigated at each time point. We first captured an image in traditional power Doppler mode and then performed contrast-enhanced imaging; this was video recorded for 5 minutes. The total dose of the contrast agent was approximately 0.32 mL (6.4×10^8 microbubbles), and this was injected through each rabbit's large veins in the ears, followed by 1.5 mL of a normal saline flush. The video was analyzed off-line, frame-by-frame, and a freely downloaded software, KM player (KMPlayer_EN_3.0.0.1442) was used to retrieve the appropriate images. We selected