INTRODUCTION

Healing at the tendon-bone interface is an important component in anterior cruciate ligament reconstruction as well as rotator cuff and Bankart lesions in the shoulder. Poor or slow healing can hinder post-operative rehabilitation, return to sport or normal activities and ultimate clinical outcome. Low-intensity pulsed ultrasound (LIPUS) is clinically successful in the healing of fresh fractures and established non-unions [1-4]. Busse et al., [4] using meta-analysis recently reported that LIPUS may significantly reduce the time to fracture healing for fractures treated non-operatively. LIPUS also plays a positive role in bone healing in callus distraction, bone ingrowth into porous devices, as well as cartilage defect healing. Takakura et al., [5] recently reported LIPUS to enhance the early healing of medial collateral ligament injuries. We hypothesized that low-intensity pulsed ultrasound may enhance tendon-bone healing following anterior cruciate ligament reconstruction. This pilot study evaluated the effect of LIPUS on tendon-bone healing in an intra-articular sheep model.

METHODS

An open intra-articular reconstruction was performed in 21 adult sheep using an extensor tendon model following ethical approval (01/08). An Endobutton was used for femoral fixation and the tibia secured over an osseous post. Animals were allocated to 2 groups (Control or Low-Intensity Pulsed Ultrasound) and killed at 3, 6 and 12 weeks (n=2 for the controls and n=5 for the LIPUS group). LIPUS treatment (200 µsec burst of sine waves at 1.5 MHz repeated at 1 kHz, 30 mW/cm2) was performed daily for 20 minutes on the lateral aspect of the femur and anteromedial aspect of the tibia with ultrasound gel. Computed tomography (CT) was performed at sacrifice using a Toshiba Scanner (Tokyo, Japan) and the quality of the interface graded. The femoral and tibial bone tunnels and intra-articular portion of the tendon were fixed in formalin. The bony tunnels were decalcified in formic acid/formalin and embedded in paraffin. Serial sections of the tunnels and intra-articular tendon were stained with H&E. Histology was graded in a blinded fashion at 4 quadrants within the tendon-bone tunnels and analysed using the Mann-Whitney ANOVA. Immunohistochemical staining and immunofluorescence for VEGF and Smad 4 was performed using standard techniques.

RESULTS

All animals recovered uneventfully following surgery and were allowed immediately weight bearing. No adverse events were noted during the LIPUS treatment. CT scans revealed marked increase in signal intensity along the entire tendon-bone interface on the femoral and tibial sides in the LIPUS group compared to control.

Histology demonstrated a progression in healing in the controls and LIPUS treated group with time. Histological grading of the LIPUS treated animals was superior to control animals at all time points (p<0.05). The tendon-bone interface in the control animals at 3 weeks was characterized by loose connective tissue with minimal new vascularity. The LIPUS treated animals, on the other hand, had a significant number of new blood vessels at the interface as well as a more cellular appearance of the tendon and new blood vessels within the tunnel.

New connective tissue was also observed between the fascicle bundles of the tendon at 3 weeks. Marked differences in vascularity between the controls and LIPUS treated was observed at 6 weeks and continued at 12 weeks (figures 1-3). New bone was noted to be streaming into the tendon graft itself in the femoral and tibial tunnels at 6 weeks in the LIPUS treated group (Figure 2). By 12 weeks a mature tendon-bone interface had developed in the LIPUS treated samples (Figure 3) that were superior to controls. Immunostaining revealed increased expression levels for VEGF and Smad 4 in the LIPUS treated group compared to controls at all time points. VEGF expression was still present at 12 weeks in the LIPUS treated group, however expression in the controls was barely detectable.

DISCUSSION

Methods to improve tendon-bone healing may provide a new avenue of treatment and rehabilitation protocols following anterior cruciate ligament reconstruction. A molecular basis for LIPUS treatment mediated by intracellular calcium signaling has been reported for fracture healing [6]. LIPUS stimulates the production of angiogenic factors in-vitro [7]. This study supports that LIPUS does indeed influence tendon-bone healing in an intraarticular sheep model. Noted vascularity was observed in the LIPUS treated samples earlier and in a superior fashion compared to controls. New bone formation at the interface with Sharpey’s fibres was observed as early as 6 weeks in the LIPUS treated samples. The improved vascularity was accompanied with increased expression of VEGF and Smad 4. These data suggest that LIPUS may not only influence vascularity through VEGF expression but influence signal transduction mediated through a Smad pathway. The effect of LIPUS on the mechanical properties of this interface is currently under examination.

REFERENCES


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