BIOMECHANICAL AND HISTOLOGICAL EVALUATION OF THE EFFECT OF LOW INTENSITY PULSED ULTRASOUND TO INTERFACE HEALING BETWEEN BONE AND TRANSPLANTED SOFT TISSUE GRAFT USING A NEW ANIMAL MODEL

INTRODUCTION: The successful outcome of anterior cruciate ligament reconstruction using a soft tissue graft mainly depends on the biological anchoring of the tendon graft transplanted into the bone tunnel. To clarify the actual interface strength throughout the entire integration process, we developed a new animal model using a tendon graft reinforced with a suture material (1). Since the actual interface strength is evaluated until the complete integration, this model is appropriate for evaluation of the effect of biological treatments such as gene therapy and administration of a growth factor to accelerate the integration of the tendon-bone interface. Although the effect of biological treatment has been reported in many papers, it is difficult to apply it in the clinical situation because of the lack of established delivery systems of growth factors. In contrast, low intensity pulsed ultrasound (LIPUS) therapy is popular for the treatment of fractures without major complications, furthermore, some authors reported that the remodeling of the injured ligament and the integration process between the bone and the tendon graft were enhanced by LIPUS (2,3,4). The purpose of this study was to evaluate the effect of LIPUS on the integration process of the tendon graft to the bone in our new animal model.

MATERIALS AND METHODS: Thirty-two skeletally mature female Japanese White Rabbits (3.5-4.0Kg) were used for this study, which was carried out in accordance with the Guidelines for Animal Experimentation, Hirosaki University. The extensor digitorum longus (EDL) was detached from the femoral insertion. A No.2 Ethibond suture (Ethicon, Inc., Somerville, New Jersey, USA) was penetrated into the mid-substance of the EDL, and the muscle-tendon junction and the free end of the EDL were lock-sutured. A bone tunnel 2.5mm in diameter was drilled on the proximal tibia perpendicular to the longitudinal axis and the length of the bone tunnel was measured. The graft was drawn through the bone tunnel, and was tightly fixed at the medial cortex of the tibia with a button. The bilateral limb underwent the same procedures. After surgery, all animals were allowed unrestricted in their cages without immobilization. LIPUS (a 200 microsecond burst of sine waves at 1.5MHz, repeated at 1KHz, 30mW/cm²) therapy was performed daily for 20 minutes on the anterolateral aspect of the tibia to the bone tunnel with ultrasound gel under general anesthesia on one limb of each animal (LIPUS group). The contralateral limb was not exposed to LIPUS (control group). Eight animals each were killed at 4, 6, 8 and 12 weeks after surgery (6 for biomechanical testing and 2 for histological evaluation). For biomechanical testing, the specimen was mounted on the material testing machine (Instron 4465; Instron Corp., Canton, Massachusetts, USA) with a specially designed clamp to apply an axial tensile loading along the longitudinal axis of the bone tunnel. After the removal of the fixation button, the ultimate failure load was measured at a crosshead speed of 100 mm/min. Because the length of the bone tunnel varied between specimens, the failure load-to-tunnel length ratio (FTR) was determined by normalizing the failure load by the tunnel length. A paired t-test was used to compare the biomechanical data between the two groups at each time point. One-way analysis of variance with the Scheffé test for post hoc was used to compare the biomechanical data at different time points within each group. The level of significance was set at p<0.05. The specimens were prepared for histological evaluation of the tendon-bone interface using hematoxylin and eosin, and Masson’s trichrome staining.

RESULTS: Biomechanical testing

In the control group, all tendon grafts were pulled out of the bone tunnel at each time period. In the LIPUS group, all tendon grafts were pulled out of the bone tunnel at 4, 6, and 8 weeks. At 12 weeks, failure mode was mixed with pull out and partial pull out. FTR was significantly larger in the LIPUS group compared to the control group at each time period. In the LIPUS group, significant increases of the FTR were observed at 12 weeks compared to 4, 6, and 8 weeks. In the control group, there were significant increases of the FTR at 8 and 12 weeks compared to 4 and 6 weeks (Figure 1).

Control
LIPUS

Figure 1: Failure load-to-tunnel length ratio of both groups

DISCUSSION:

Walsh et al. recently evaluated the effect of LIPUS on the integration process of the tendon graft to the bone and demonstrated that the improved vascularity was accompanied with increased expression of vascular endothelial growth factor (VEGF). On the other hand, they reported that it was difficult to evaluate the strength of tendon-bone interface after the weakest point shifted from tendon-bone interface to the graft mid-substance. Our experimental model may allow the biomechanical evaluation of the entire integration process including the period after the weakest point shifts. In our biomechanical testing, all tendon grafts pulled out of the bone tunnel showed actual interface strength enhanced by LIPUS. Furthermore, in the histological evaluation, we observed acceleration of the tendon-bone interface by LIPUS such as more abundant vascularity in the LIPUS group compared to the control group. This result indicated that LIPUS accelerated the integration process in the early phase.

REFERENCES:
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