INTRODUCTION

Low-intensity pulsed ultrasound stimulation (LIPUS) effects are reportedly derived from the promotion of cell differentiation, which induces acceleration of fracture healing, resulting in earlier restoration of strength at the fracture healing site.

Previous studies have attempted to determine which stages of fracture healing are affected by LIPUS. Many basic studies suggested that LIPUS affected the inflammation, angiogenesis and soft callus formation stages. However, several studies suggested that LIPUS had no promoting effect in hard callus formation and remodeling stages [1]. Most previous basic studies have investigated the effects of LIPUS by histological evaluation, which is useful in clarifying cell differentiation and the formation of tissue morphology/arrangement, but is limited to observation in a 2-dimensional (2D) plane. Another method adopted has been mechanical testing of harvested specimens, but this is limited in that the testing is destructive and the strength of the healing site is evaluated in only one of multiple spatial planes.

In recent years, several basic studies have investigated fracture healing site morphology using micro-computed tomography (µCT). The advantage of µCT lies in the non-destructive morphological and densitometric assessments in 3-dimensional (3D) planes. In evaluating the effects of LIPUS on fracture healing by means of µCT, assessment is only possible after the healing stage has formed mineralized fracture callus. The purpose of the present study was to quantitatively evaluate the effect of LIPUS on bone healing by means of µCT throughout both modeling and remodeling processes using a gap healing model in rabbits.

MATERIALS AND METHODS

Surgical Procedures

A total of 42 skeletally mature between 21 and 23-week-old male Japanese white rabbits (Kitayama Labs, Nagano, Japan), weighing 3.4-4.0 kg, were used for this study. Under general anesthesia, four transfixation pins (diameter, 2 mm; length, 50 mm) were inserted at the metaphyseal regions of the tibia in the frontal plane using a custom-made surgical pin driver. Transverse osteotomy was performed using a r-saw (blade thickness, 0.36 mm) with continuous irrigation with saline solution across the mid-shaft of the tibia at 12 mm distal to the tibio-fibular junction. The osteotomy with a 2-mm gap was immobilized with four pins fixed to an external fixator with double side bars. All procedures were performed in accordance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

LIPUS Treatment

The LIPUS system (model SAFHS®20001, Teijin Pharma, Tokyo, Japan), which transmits 200-µsec burst of 1.5-MHz sine waves repeated at 1kHz with an average intensity of 30mW/cm², was used. After postoperative day 3, LIPUS was continued under general anesthesia for both the treatment group (n=7/group/time point) and the control group (n=7/group/time point). The transducer was placed onto the anterior surface of the operated leg with ultrasound coupling gel, for 20 min, six times/week, for 4, 6, or 8 weeks. The control group also received a sham inactive transducer under exactly the same condition as the LIPUS group.

µCT Analysis

All animals were euthanized with CO₂ asphyxiation and the entire right tibia was removed. After removal of soft tissues, the harvested tibia were scanned by µCT (Scan X mate-E090; Comscantecno, Kanagawa, Japan). The scan was performed along the long axis of the diaphysis, with a voltage of 60 kVp and a current of 80 µA. Scan range covered 5 mm proximal and 5 mm distal to the center of the gap, with a resolution of 28.57 µm³ voxel size. The region of interest (ROI) was set at the callus healing area (Fig. 1) defined by the gap filled with callus in 2D-CT scans and extended 0.5 mm proximally and distally from the center of the osteotomy gap with a total of 36 CT axial scans. 3D reconstruction of mineralized tissue was performed using a TRI-BONE system (Ratoc System Engineering, Tokyo, Japan). A threshold for newly formed mineralized callus was set as 200 mg/cm³ [2].

Morphometric parameters used for evaluation were mineralized callus volume (BV, cm³) and volumetric bone mineral density of mineralized tissue comprising the callus (mBMD, mBMD = BMC/BV, mgHA/cm³). The whole ROI was measured and was subdivided into three zones (Fig. 1): a periosteal callus zone (External; red zone); a medullary callus zone (Endosteal; green zone); and the cortical gap zone as the remaining zone (Inter cortical: yellow zone). For each zone, BV and mBMD were measured.

Statistical Analysis

The µCT evaluations were analyzed using a one-way ANOVA test. If that correction did not achieve normality, then a Kruskall-Wallis ANOVA on ranks was utilized. Data were all presented in mean and standard error of the estimate. Values of p<0.05 were considered statistically significant.

RESULTS

Callus Volume

The results of BV were shown in Fig. 2. The control groups showed no significant differences. However, in the LIPUS groups, mean BV for the Endosteal zone was significantly lower for the 8-week group than for the 4-week group (p < 0.05). Comparing results at the same time point, the LIPUS group at 8 weeks was significantly higher than that of the control group in the Intercortical zone (p<0.001).

Volumetric Bone Mineral Density of Mineralized Tissue

The results of mBMD were shown in Fig. 3. The control groups showed no significant differences. However, in the LIPUS groups, the 8-week group was significantly higher than the 4-week group for Total, External, Internal, and Endosteal zones, respectively (p < 0.001, 0.001, 0.001, and 0.05). Comparing results at the same time point, the LIPUS group at 8 weeks was significantly higher than that of the control group in both External (p < 0.05) and Intercortical (p < 0.05) zones.

DISCUSSION

The current investigation focused on the morphology of the gap healing site under the influence of LIPUS by quantifying changes in the localization of newly formed callus. The most striking finding in our study was that LIPUS accelerated bone formation in the External and Intercortical zones and callus resorption in the Endosteal zone. Therefore, LIPUS enhanced corticalization and medullary canal formation at the gap healing model, which could lead to earlier restoration of the structural integrity of the healing site.

REFERENCES


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