LIPUS enhances fracture healing at critical angles

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INTRODUCTION

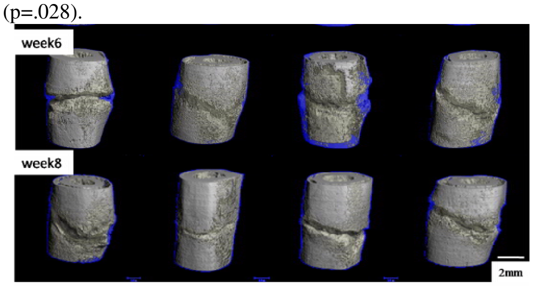
Low-intensity pulsed ultrasound (LIPUS) is one of the promising treatment methods to accelerate fracture healing by providing mechanical stimulation on bone [5]. Our previous studies also indicated stimulatory effects of LIPUS on human periosteal cells [8] in vitro and complex fracture healing clinically [7]. Our clinical study has provided clues that the stimulatory effect of LIPUS was not just localized to the area of direct application [7]. Most common forms for ultrasound to travel in a solid material are longitudinal and shear waves. When the longitudinal wave travels between two materials, such as soft tissue and bone, with different impedance at an oblique angle of incidence, part of the longitudinal waves converse into shear waves at the interface [6]. This suggested that the combination of the longitudinal wave and the shear wave might be one of the key factors of the efficacy of the LIPUS treatment on accelerating the fracture healing. The physical properties of ultrasound show that, ultrasound transmitted at the first or second critical angles can maximize the longitudinal or shear waves along the interface respectively [1], and hence, maximizing the ultrasound energy on the fracture site. We therefore hypothesized that the treatment effect on fracture healing of LIPUS might be further enhanced with application at a critical angle by maximizing the biological effects on periosteum. In this study, we investigated the effect of LIPUS on fracture healing at different incident angles with femoral fracture in a rat model.

MATERIALS AND METHODS

A femoral fracture model in left femur of each rat was performed according to our well established protocol in 144 SD rats [4]. Fractured rats were divided into four LIPUS treated groups at four different incident angles (0°, 22°, 35° and 48°) (n=11/group/time point). Fractured rats were held individually on a standard platform with different angle holders (0°, 22°, 35°and 48°) connected to the LIPUS device (Exogen 2000®, Smith & Nephew Inc. Memphis, TN), which transmits 200-µsec burst of 1-MHz sine waves repeated at 1 kHz with an average intensity of 30mW/cm^2 for 20min/day and 6 days/week. Radiographies were taken weekly post-treatment in a cabinet X-ray system model 43855C for routine monitoring of the fracture healing. The callus width and area of all the X-ray films were measured by the Metamorph Image Analysis System. In radiographic qualitative analysis, the completed mineralized callus bridging percentage was examined by two blinded orthopaedic surgeons according to a standardized scoring system. At different time points (week 2, 4, 6 and 8) after euthanasia of the rat, the fractured femur was harvested for studying the bone microarchitecture of the healing femur by µCT machine (µCT 40, Scanco Medical, Zurich, Switzerland). Part of the specimens (n=3/group/time point) were then proceeded for decalcified histology and stained with Hematoxylin & Eosin (H&E) while others (n=8/group/time point) were undergone mechanical torsional test after densitometric measurement. One-way analysis of variance (ANOVA) with significance level at 0.05 was done for statistical analysis.

RESULTS

For the qualitative radiographic analysis, about 92% of 35° group samples callus bridged in week 8 and it was nearly 40% more than the 0° group. Quantitatively, the callus area of 35° group was larger than all other groups at earlier time points from week 2 to 4 and significantly larger than 22° group in week 2 and 3 (p=0.013, 0.007). The other two groups, 0°and 48°, showed a larger callus area than that of the 22° group at all the time points but did not show any statistical significance. The radiographs, microCT 3D reconstructed images and histomorphology of the fractured femora in 35° group also showed a faster obliteration of fracture line at the fracture site than all other groups after week 6 (Fig.1). For the quantitative microCT analysis, more advanced callus mineralization of 35° group was also demonstrated in its high-density bone volume (BVh) than all other groups in week 6. Interestingly, the low-density bone volume (BVl) of 48° group reached its peak in week 4, during which BVl was significantly larger than all other groups. For histomorphological quantitative analysis, the osseous tissue increased rapidly in 35° group from week 6 to 8 and reflected in the significantly high osseous tissue area to cartilaginous tissue area ratio than all other groups in week 8 (p<.02). The torque at bone failure of 35° group was higher than all other groups, especially 0° (p=.041) and 22° groups (p=.028).



G(0°) Y(35°) R(22°) B(48°)

Fig.1 The representative 3D reconstructed images showing the low (Blue shaded area) and high (grey shaded area) density bone volumes of all the LIPUS treated groups at different angles in week 6 and 8.

DISCUSSION

This study showed that LIPUS transmitted at 35° significantly accelerated the fracture healing by accelerating callus bridging, enhancing more advanced mineralization of callus and increasing biomechanical properties when compared to other treatment groups, especially 0° group. These suggested that 35° may be a critical application angle of LIPUS in our rat model. The combination of the ultrasound longitudinal and shear waves at critical angle may maximize the biological effect because most of the ultrasound waves propagated either along or through the fracture site. Most of the ultrasound energy could reach the fracture site at the first critical angle. Our findings substantiated Azuma's work showing that the fractured group treated with LIPUS for longer period of time indicated a more extensive callus bridging in both radiographical and histological qualitative analysis and significantly higher maximal torque than those treated with LIPUS for shorter period [1]. Our study also found that LIPUS transmitted at 35° showed even more advanced callus bridging and significantly high torque at bone failure than LIPUS transmitted at normal angle 0°. Thus, Azuma's study [2] was also consistent with our study that maximizing LIPUS energy on fracture site may further enhance fracture healing. Moreover, transmitting LIPUS at 35° may indirectly increase the dose of the ultrasound energy, which was maximized at the critical angle, reaching on the periosteum. Therefore, this may further stimulate periosteal cell proliferation, differentiation, and osteogenic activities with a dose-dependent manner [8], resulting in enhanced mineralization of fracture healing [8,9] and hence accelerate the fracture healing process [3,8]. In conclusion, LIPUS transmitted at 35°, the critical angle in our rat femoral fracture model, enhances the fracture healing by maximizing the combinations of longitudinal and shear waves. This study, more importantly, demonstrated the potential of LIPUS on fracture healing that could be further enhanced by manipulating the basic physical properties of ultrasound waves.

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