LOW-INTENSITY PULSED ULTRASOUND ACCELERATES RAT FEMORAL FRACTURE HEALING BY ACTING ON THE VARIOUS CELLULAR REACTIONS IN THE FRACTURE CALLUS

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Relevance to Musculoskeletal Conditions:
Fracture healing can be accelerated by exposure to low-intensity pulsed ultrasound (LIPUS) in both animal fracture models and clinical trials,[1-3,5] but the mechanism of action has been unclear yet. The primary question addressed in this study was whether the accelerating action of LIPUS on fracture healing is based on the stimulation of a specific cellular reaction involved in the healing process.

Introduction:
We had found the accelerating effects of daily LIPUS-treatment up to 21 days after the fracture on the fracture healing using a rat closed femoral fracture model. Because in this model, a series of stage-specific cellular reactions were observed until repair, we investigated whether the beneficial effects of LIPUS depended on the duration and timing of LIPUS-treatment in this same model to determine the target reaction of LIPUS in the fracture healing process in vivo. In the present study, we divided the interval up to 25 days after the fracture into three periods (Ph-1, Ph-2, Ph-3), which involved various essential reactions for the fracture healing, and investigated the effects of partial treatment with LIPUS on the healing process. If the advanced effects of LIPUS on fracture healing are based on the action toward a specific reaction, the efficiency of acceleration should depend on the timing of the partial LIPUS-treatment. In last ORS meeting, we showed that partial LIPUS-treatment was able to induce the beneficial effects on the rigidity of fracture callus. In this study, we investigated the relationship between the timing of the partial LIPUS-treatment and the efficiency of accelerating action by estimating mechanical properties, bone mass, histology, and the microstructure of the callus, the latter by using the three dimensional microfocus X-ray CT technique.

Materials and Methods:
Ten-week-old Long-Evans male rats were used, and the fracture models were produced by the method previously described[4]. LIPUS was generated with the transducer (effective area 3.88 cm²) of the Sonic Accelerated Fracture Healing System (SAFHSR, Exogen Inc.) under the following conditions: frequency; 1.5 MHz, pulse repetition frequency; 1.0 kHz, pulse burst width; 200 msec, and intensity; 30 mW/cm² (SATA). The femoral fracture site of anesthetized rats was exposed to LIPUS once daily for 20 minutes. The right femur of the animals was exposed to LIPUS once daily for 20 minutes. The effect of LIPUS was evaluated. To observed the effect of LIPUS in progress on day 9 and day 17 after fracture, we sacrificed three rats in Ph-1 and in Ph-2 groups. To evaluate the bone bridging at the fracture site by use of a three-dimensional (3D) reconstruction technique, we took 50 tomograms extended on either side of the fracture line.

Results:
The torsional strength (maximum torque) of the femur treated by LIPUS was significantly higher than on the control side in all groups. The torsional strength of the femur treated by LIPUS in the T-group was higher than those in the Ph-1, Ph-2, or Ph-3 group. No significant differences in BMC in the area near the fracture site were observed between the LIPUS-treated side and the control side in any of the groups. The fracture line of the LIPUS-treated femur was bridged by new bone formation three-dimensionally in all groups. In contrast, bridging of the fracture site by new bone formation did not occur substantially in the control femur. Histological evaluations showed that early endochondral ossification on day 9, and late endochondral ossification on day 17 in the LIPUS-treated femur were greater than that in the control. Furthermore, more extensive bone bridging was observed in the LIPUS-treated femur than in the control femur in not only the T group but also the Ph-3 group on day 25.

Discussion:
Torsional strength of the femur was increased as a result of LIPUS treatment in all groups. This result suggests that partial LIPUS-treatment is able to accelerate fracture healing regardless of its timing. Additionally, torsional strength in the T group was higher than those in the other three groups, probably because multiple reactions were accelerated by continuous treatment, and its effects were intensified. In the results for the multiple viewing from various angles of 3D-analysis, union and microstructure of trabecule in the fracture callus were advanced in LIPUS-treated femurs than in the control. This result demonstrates that LIPUS advances the onset of bone bridging in fracture healing. In the histological analysis, endochondral ossification was more advanced in LIPUS-treated femurs on day 9 and 17 than in the control. Since a number of reactions occur before and/or during endochondral ossification, there are several indications of possibility that LIPUS stimulated several reactions in those stages. The partial LIPUS-treatment only in Ph-3 was effective in advancing endochondral ossification and bone remodeling. This results indicates that the stimulation of bone remodeling by LIPUS in the Ph-3 period contributes the accelerating effect LIPUS on fracture healing.

Conclusion:
Though we could not define the main target, we found out that LIPUS accelerated the rat femoral fracture healing regardless of the timing of LIPUS-treatment. LIPUS thus appears to act on various cellular reactions involved in the fracture healing process.

References:
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