Low-Intensity Pulsed Ultrasound Stimulates Intramedullary Bone Formation and Homing of Circulating Connective Tissue Progenitors at Fracture Site with Periosteal Stripping in Mice.

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INTRODUCTION

Delayed union or nonunion is the most common complication in fracture treatment. To enhance the fracture healing, a variety of treatment techniques have been developed. Low-intensity pulsed ultrasound (LIPUS) therapy is one of the beneficial procedures for patients to be noninvasively treated at the fracture site. Despite clinical and experimental studies demonstrating the enhancing effect of LIPUS on bone regeneration, the biophysical mechanisms involved in the complex fracture healing process remain unclear and require further research. Recent investigations have shown osteogenic connective tissue progenitors present in the systemic circulation and contribute to the fracture healing. Recruitment of progenitor cells through systemic circulation may have therapeutic potential for fracture healing. We hypothesize that LIPUS stimulates homing of circulating osteogenic progenitors to the fracture site and enhances osteogenesis in the progenitor deficient sites.

METHODS

All animal procedures were approved by the Institutional Animal Care and Use Committees of our institution. Transgenic GFP C57BL/6-TgN and wild-type C57BL/6 mice were surgically conjoined for parabiosis at 12 weeks old. Partners achieve equal blood chimerism of GFP cells within two weeks. Three weeks after parabiosis surgery, a transversal fracture of diaphysial femur was created with circular saw in contralateral hind limb of wild-type partner. To create a "challenged" fracture, periosteum is stripped circumferentially from osteotomy site to both joints. Fracture was stabilized with an intramedullary pin. Fracture site was treated with daily exposure of LIPUS for 20 minutes. LIPUS treatment was not applied to control group. Fracture site was harvested at 2 and 4 weeks. Samples were cryosectioned for histological evaluation. Sections were stained with alkaline phosphatase (AP) for identification of osteogenic differentiation and hematoxylin and eosin for qualitative assessment. Histological images were digitally captured under bright-field and fluorescent microscopy. Image quantification was performed using Image-Pro Plus software (Media Cybernetics).

RESULTS

Histological findings showed poor callus formation around fracture gap in both group. Periosteal new bone formation did not occur in the extramedullary region of fracture site. Instead, fibrous or cartilaginous tissue was present. In LIPUS group, new bone formation tended to be observed in the intramedullary site close to fracture gap. AP activity was expressed at the bone forming site, especially intramedullary space near fracture gap (Fig.1). In contrast, control group displayed less bone formation in the marrow space. Area of AP expression was analyzed at area of interest, to assess intramedullary bone formation with the relative depth from the fracture gap. LIPUS-treated group resulted in significantly greater AP expression in the gap site (0-0.5mm from gap center) at 2 and 4 weeks post fracture and in the adjacent site (0.5-1.0mm from gap center) at 2 weeks post fracture than control group (Fig.2). GFP+ cells gaining access to the fracture site via systemic circulation were identified in the intramedullary space close to the osteotomy site (Fig.3). Co-localization of GFP and AP expression was confirmed for osteogenic potential.

DISCUSSION

This study demonstrated that bone formation was stimulated by LIPUS exposure in the intramedullary site of mice femur fracture with periosteal stripping. Data also suggested that LIPUS induced homing of circulating osteogenic progenitors to the fracture site for possible contribution to new bone formation. In this study, periosteum was removed around femur osteotomy site to assess the effect of LIPUS in an impaired fracture healing. In a setting in which the local progenitor population is deficient, delivery of osteogenic progenitor cells and performance of bone formation are limited through the fracture healing process. Only bone marrow derived cells in local site could be expected to react to LIPUS stimulation. Homing of osteogenic progenitors from systemic circulation may compensate performance of local progenitors through intrinsic biological process, although further study is necessary to confirm and extend these findings.

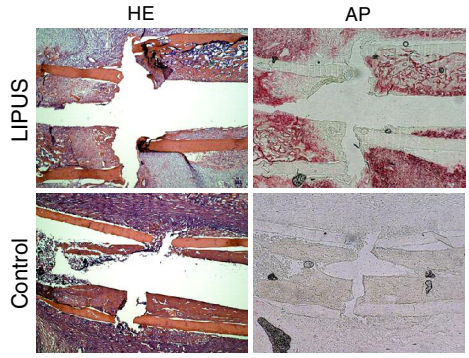

Figure 1. Bright-field micrographs of fracture site two weeks after fracture.

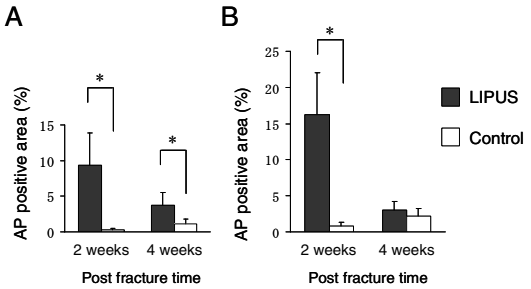

Figure 2. Percentage of AP positive area in the intramedullary space of the fracture site. (A) gap site; 0-0.5mm from gap center (B) adjacent site; 0.5-1.0mm from gap center. Values are presented as mean ± SEM (N = 5). *p<0.05 for difference between treatment groups.

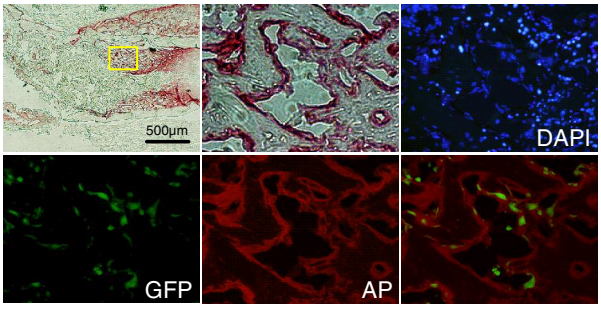

Figure 3. Fluorescent micrographs of fracture site in LIPUS group two weeks post fracture.

ACKNOWLEDGEMENT

This work was supported by the Grant-in-Aid for Scientific Research from Ministry of Education,Culture, Sports, Science and Technology of Japan (#20591763).

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