Low Intensity Pulsed Ultrasound Accelerates Fracture Healing through SDF-1/CXCR4 pathway

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INTRODUCTION

Low intensity pulsed ultrasound (LIPUS) was proven to promote fracture healing. However, the underlying mechanism through which LIPUS enhances the fracture healing is still not fully depicted. Mesenchymal stem cells (MSCs) are multipotent cells which can migrate to the fracture site and play a key role in fracture healing process. This migration effect is mainly modulated by stromal cell-derived factor 1 (SDF-1) /CXCR4 pathway [1]. Our previous work has shown that LIPUS directly increases SDF-1/CXCR4 expression in MSCs and also induces MSCs migration in vitro [2]. In this study we investigated the hypothesis that LIPUS would improve fracture healing through enhanced MSCs recruitment via SDF-1/CXCR4 pathway in a rat femoral fracture model.

MATERIALS AND METHODS

Thirty 8-week old female Sprague-Dawley rats were used in this study. Closed transverse fracture was performed in the right femoral shaft. Rats were randomly assigned to three groups: Group 1 (LIPUS, n=10) received LIPUS treatment for 20 minutes per day, 5 days per week; Group 2 (LIPUS+AMD, n=10) were injected with AMD3100 (1mg/kg/per day, intraperitoneal) while receiving the same LIPUS treatment as group 1. Group 3 served as Control without treatment. Green fluorescent protein labeled MSCs (GFP-MSCs) obtained from Cyagen (Cyagen, Guangzhou, China) were used to investigate MSCs migration during fracture healing. 1x10^6 GFP-MSCs were administered via left ventricle to all animals on post-surgery day one. Lateral radiographic analysis was performed weekly for the quantitative measurements of callus width and area.

After 4-weeks of treatment, all the animals were euthanized and the fractured femurs were carefully collected for micro-computed tomography (micro-CT) and four-point bending biomechanical test. Ex vivo assessment of MSC localization was performed using bioluminescence imaging system (IVIS 200, Xenogen, US). GFP signal intensity of the callus area was measured and normalized to that of the contralateral area in left femur by the use of Living Image Software version 3.0 (Xenogen Corp., USA). One-way ANOVA was used to compare the statistical differences among the above measurement variables, followed by Tukey post-hoc test. Statistical significance level was set at p<0.05.

RESULTS

Radiological assessment: Callus width in LIPUS group were found to be significantly larger by 26.8% at 1 week (p=0.031), by 33.6% at 2 weeks (p=0.01) and by 35.0% at 3 weeks (p=0.07) post-fracture than Control group, respectively. Callus width in LIPUS group was significantly larger by 27.8% at week 2 (p=0.035) and by 30.0% at week 3 (p<0.022) post fracture than LIPUS+AMD group. Callus area was significantly larger in LIPUS group by 55.1% at 1 week (p=0.002), by 55.5% at 2 weeks (p=0.002) and by 64.0% at 3 weeks (p=0.032) than Control group, respectively. Callus area in LIPUS group was significantly larger by 37.7% at 2 weeks (p=0.047) than LIPUS+AMD group. Both callus width and area in the LIPUS+AMD group were shown to be larger than Control group in 1 week, 2 weeks and 3 weeks post-fracture without significant difference. (Figure 1)

Mechanical testing: At week 4, In LIPUS treatment group, the ultimate load was significantly higher by 100.7% (p<0.0001) and 30.2% (p=0.025) than Control group and LIPUS+AMD group, respectively; the energy to failure was marginal higher by 108.3% (p=0.061) than Control group. In LIPUS+AMD group, the ultimate load was significantly higher by 54.2% than Control group (p=0.01). (Figure 2)

Micro-CT analysis: The quantitative measurement of the total callus volume (TV) and the total mineralized callus volume (BV) did not show statistical significant difference at week 4 among three groups. Bone mineral density (BMD) in LIPUS treatment group was found to be significantly higher by 10% than LIPUS + AMD group (p=0.006) and by 14.5% than Control group (p=0.0053). (Figure 3)

GFP signal intensity analysis: The ex vivo relative GFP intensity in LIPUS group was significantly increased 4.49 times than LIPUS+AMD group (p=0.01), and increased 1.43 times than Control group (p=0.083). (Figure 4)

DISCUSSION

SDF-1 was reported to modulate homing and engraftment of circulating MSCs by binding to its receptor CXCR4, thus expanding the pool of osteogenic precursor cells in fracture site [3]. This study demonstrated that LIPUS promoted MSCs migration and improved bone fracture healing in vivo, which is consistent with our previous findings. This promoting effect was attenuated by CXCR4 specific inhibitor, AMD3100. Callus formation was generally decreased in LIPUS+AMD group than LIPUS group, shown by smaller callus width and area from the radiographs. Bone remodeling process was also disturbed in the presence of AMD3100, shown by significantly lower ultimate load and lower energy to failure than LIPUS group from the mechanical testing. Bone mineralization was affected in LIPUS+AMD group, which was indicated by significantly lower bone mineral density from micro-CT analysis than LIPUS group. AMD 3100 prohibited MSCs migration to the fracture site promoted by LIPUS, which was indicated by lower GFP signal measured in the callus area of LIPUS+AMD group. The findings of this study showed that mechanical stimulation, in form of LIPUS, might be able to modulate SDF-1/CXCR4 pathway in MSCs. It also provides strong evidence that SDF-1 mediated MSCs migration might be one of the crucial mechanisms through which LIPUS enhances fracture healing.

SIGNIFICANCE

This study helped us understand the mechanism of LIPUS for promoting fracture healing in the clinical settings.

REFERENCE

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