Low Intensity Pulsed Ultrasound Increases Stromal Cell-Derived Factor 1 Signaling in Rat Mesenchymal Stem Cells

Wei, FY; Leung, KS; Qin, L; Sun, MH; +Cheung, WH
+Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China.
louis@ort.cuhk.edu.hk

INTRODUCTION
It has been well documented that low intensity pulsed ultrasound (LIPUS) promotes fracture healing. Currently this safe biophysical intervention has been approved by the Food and Drug Administration for the treatment of fresh fractures and non-unions. However, the underlying cellular and molecular mechanism through which LIPUS enhances the fracture healing is still unknown. Mesenchymal stem cells (MSC) are multipotent cells which can migrate to sites of injury and promote structural and functional repairs in many organs including bones. This migration effect is mainly modulated by stromal cell-derived factor 1 (SDF-1) through its specific receptor, CXCR4. Previous study has shown that LIPUS promotes the synthesis of interleukin-8 (IL-8) in osteoblasts and blood mononuclear cells [1], which is associated with SDF-1/CXCR4 pathway [2].

Therefore, we hypothesize that LIPUS promotes MSCs migration through SDF-1/CXCR4 pathway.

MATERIALS AND METHODS
MSC isolation & culture: MSCs were isolated from 6-week female Sprague Dawley rats. Briefly, bone marrow was collected from bilateral femurs under aseptic conditions and expanded in α-MEM containing 10% FBS and L-glutamine. After grown to confluence, cells were seeded in six-well plates at 2 x 10^5 cells/well.

LIPUS treatment: LIPUS was provided by a sonic Accelerated Fracture Healing System (SAFHS; Smith & Nephew, Memphis, TN, USA). The 6-well culture plate was placed on the ultrasound transducer with a thin layer of coupling gel. LIPUS treatment (30 mW/cm^2, intensity at 1.5 MHz) was given through the bottom of the culture plate for 20 minutes daily at 37°C for 5 days.

Real-time RT-PCR Analysis: After 5 days treatment, total RNA was isolated from the MSCs in LIPUS treatment group and no-treatment Control group with Qiagen RNeasy isolation kit (Qiagen, Valencia, CA, USA). The RNA was reverse-transcribed and amplified by PCR. Quantitative PCR was performed using LightCycler SYBR Green Master and a LightCycler qPCR (Roche Diagnostics, Penzberg, Germany). mRNA levels of each gene of interest were normalized to GAPDH levels.

Enzyme-linked immunosorbent assay: The culture medium was collected from LIPUS treatment group and Control group after 5 days treatment. Protein level of SDF-1 was quantified using Quantikine SDF-1α enzyme immunoassay kit (R&D System, USA) according to the manufacturer's instructions. The optical density of each well was determined using a microplate reader set at 450nm wavelength.

Cell migration assay: Migration assays were carried out using 8 µm Millipore inserts (Millipore, ECM508, MA, USA) for 24-well plates according to the manufacturer's instructions. Briefly, first-passage MSCs (1x10^5) in 300 µl of chemoattractant-free α-MEM medium were seeded onto the upper compartment of each chamber and placed into wells containing culture medium collected from LIPUS treatment group or control group after 5 days treatment. The medium in the upper chamber was added with or without 1µM AMD3100 (Sigma, St Louis, MO, USA). The migration chambers were incubated for 18h at 37°C in a CO_2 incubator (5% CO_2). Cells which had migrated through the membrane were stained and counted.

Statistical analysis: Independent Student t-test was used to compare experimental and control mean values. One-way ANOVA was used to compare means of different groups in cell migration assay, followed by Tukey post-hoc test. Statistical significance level was set at p<0.05.

RESULTS
SDF-1 and CXCR4 gene expression: SDF-1 expression at the mRNA level was increased 1.7 times in the LIPUS group compared to Control group (p=0.005); and CXCR4 was increased 2.9 times in the LIPUS group compared to Control group (p=0.009). (Figure 1)

SDF-1 protein expression: The SDF-1 protein level was increased 1.2 times in the media collected from MSCs treated with LIPUS than from Control group (p=0.018). (Figure 2)

DISCUSSION
The findings of this study confirm that LIPUS directly increases SDF-1/CXCR4 expression at mRNA/protein levels in MSCs and also induces MSCs migration. This is the first report to demonstrate SDF-1 signaling in MSCs can be enhanced by mechanical microenvironment modulated by LIPUS.

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]. As this study demonstrated that LIPUS-enhanced migration effect was largely abolished with CXCR4 specific inhibitor, AMD3100, this provides strong evidence that SDF-1 mediated MSC migration may be one of the crucial mechanisms through which LIPUS enhances fracture healing. Further experiments to assess LIPUS effects on MSCs migration in animal fracture model are under investigation. This study helps us understand the mechanism of LIPUS effects on fracture healing via MSC homing.

REFERENCE