The Effect of Low-intensity Pulsed Ultrasound (LIPUS) Fields on Osteocyte-Osteoblast Interactions

Fung, C H; +Cheung, W H; 2Leung, K S
+ Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Shatin, Hong Kong, China, 2 Institute of Biomedical and Health Engineering, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, People’s Republic of China

ABSTRACT INTRODUCTION:
When Low Intensity Pulsed Ultrasound (LIPUS) is applied transcutaneously, fractures at different depths are exposed to different LIPUS beam positions. In our previous study, closed femoral fractured rats were treated with LIPUS at near field, mid-near field and far field. The results indicated that distinct ultrasound field might favor particular phases of fracture healing process (1). Osteocytes are believed to be mechanosensing cells in bone. It could regulate osteoblasts by secreting certain soluble factors (2). Since different LIPUS fields bear different acoustic characteristics, osteocytes at different fracture depths are therefore exposed to different characteristics of LIPUS. Hence, we hypothesized that osteocytes treated with different ultrasound field would differentially regulate osteoblast functions via soluble factors. The objective of this study is to investigate the effect of LIPUS field on osteocyte-osteoblast interactions.

METHODS:
Cell culture: 1x10^5 MLO-Y4 per well were seeded on type-I collagen coated 6-well culture dish. LIPUS (30mW/cm^2, 117mW, 1.5MHz.) at 0mm, 60mm, 130mm, sham treatment were given 20min at 37°C. The supernatant conditioned medium (CM) was collected 24 hours after LIPUS treatments. CM was diluted with α-MEM in 1:1 ratio, and was used to culture MC3T3-E1 cells for 6 and 12 hours (assess: wound healing assay); 3 and 6 days (alkaline phosphatase assay); and 4 weeks (Alizarin red calcium nodule staining). There were five groups at all endpoints: NON (plain α-MEM medium treatment); CON (osteocytes CM); 0mm (CM from near field LIPUS treated osteocytes); 60mm (CM from mid-near field LIPUS treated osteocytes); and 130mm (CM from far field LIPUS treated osteocytes).

Wound Healing assay: At 90% confluence, cells were serum starved with 0.5% fetal bovine serum (FBS) overnight. "Wound" was created by a 1 cm-linear scratching on the cells with a sterile pipette tip. The reduction in the "wound" area was analysed by Metamorph image analysis system (n=9 per endpoint).

Alkaline phosphatase (ALP) assay: Cells were lysed by lysis buffer. PNPP solution was added to each well and incubated for 30min at 37°C. After adding stop solution, the plate was measured by microplate reader at 405nm (n=3 per endpoint).

Alizarin Red Calcium nodule staining: Cells were stained with 2% Alizarin Red. Calcium nodules in culture were scanned by photo scanner. The images were quantified by Metamorph image analysis system (n=3 per endpoint).

Statistics: All results were expressed in mean±1 standard deviation. Differences among groups were compared by one-way ANOVA and post-hoc Bonferroni test. Statistical analyses were done with SPSS version 16.0 software (SPSS Inc, Chicago, IL, USA). Statistical significance was set at p<0.05.

RESULTS SECTION:
Wound healing assay: The wound area was reduced significantly faster in 130mm group than all the other groups after 6 hours treatment (p<0.05). At 12 hours post-CM treatment, both 60mm and 130mm groups showed significantly faster reduction in wound area than that of NON group (p=0.002 and p=0.006, respectively) (Fig.1).

ALP assay: The 130mm, 60mm and CON group were significantly higher in ALP activity than that of NON group at day 6 post treatment (p=0.003, p=0.005 and p=0.049, respectively). There was no significant difference among groups at day 3 post-treatment (Fig. 2).

Alizarin Red Calcium nodule staining: The 130mm group was significantly higher in calcium nodule area than that of NON group (p=0.041) (Fig. 3).

DISCUSSION:
The present findings demonstrated that osteocytes treated with different ultrasound field would differentially regulate osteoblasts via certain soluble factors. Our results demonstrated that conditioned CM collected from osteocytes treated with LIPUS beyond near field bears higher biological effects in terms of osteoblasts recruitment and ALP activity. Furthermore, far field LIPUS-osteocyte CM group had the highest osteoblasts recruitment potential and osteogenic activities. These differential biological effects were probably due to the varying ultrasound pressure profile along the ultrasound fields. Based on the beam pattern measurements using an ultrasound tank of the 30mW/cm^2 LIPUS beam, the mid-near field in LIPUS beam has a more uniform pressure profile than in near field. At the far field the ultrasound beam is more focused and it shows the maximum ultrasound pressure as compared with the near field (1). Our previous in vivo studies demonstrated that distinct ultrasound fields might favor particular phases of fracture healing process. The current findings confirm this concept and further indicate that the osteocytes exposed with varying pressure profile along the ultrasound fields might affect the secretion of soluble factors, and hence differentially regulate osteoblastic activities.

The present results implied that osteocytes are the mechanosensors. Osteocytes are able to sense the variation of ultrasound pressure profile and regulate osteoblast correspondingly. Taylor et al. suggested that mechanically stimulated osteocytes regulate osteoblastic activity primarily via gap junction (4). However, our results indicated that secretions from mechanically stimulated osteocytes also play a vital role in transducing ultrasound mechanical stimulation to osteoblasts.

In conclusion, osteocytes stimulated with different ultrasound field would differentially regulate the osteogenic activities of osteoblasts via certain soluble factors.

SIGNIFICANCE:
The presented data provides information to help clinicians to understand the mechanism when LIPUS is used to treat fractures at different depths.

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REFERENCES: