Temporal Effects of Low Intensity Pulsed Ultrasound (LIPUS) on rhBMP-2 Induced Bone Formation in a Critical Sized Segmental Defect in the Rat

1,2Angle, S R; 3Sena, K; 1,2Sumner, D R; 1Virkus, W W; 1,2Virdi, A S;
1Department of Anatomy and Cell Biology, 2Department of Orthopedic Surgery, Rush University Medical Center, Chicago, IL, 60612 USA
2Department of Bioengineering, University of Illinois, Chicago, IL, 60607 USA
+mamarjit_virdi@rush.edu

INTRODUCTION: Fracture healing is a complex process, involving a cascade of recruitment of appropriate cells and subsequent expression of the appropriate genes during the correct time frame of the healing [1]. rhBMP-2 induced bone formation goes through an similar cascade of de novo bone formation including both, endochondral and intramembranous ossification, simultaneously [2]. As a result, multiple stage-specific cellular interaction are evident between endothelial cells, fibroblasts, osteoblasts, chondrocytes, osteocytes and osteoclasts; all of which respond to mechanical stress or mechanical strain. It has also been suggested that LIPUS is able to act on some cellular reactions involved in each phase of the fracture healing process [3]. We have previously established by qualitative, quantitative and functional endpoints that LIPUS is able to enhance healing in critical sized femoral segmental defects by stimulating two exclusive mechanism of rhBMP-2 induced bone formation, osteogenesis and callus maturation. Here, we investigate whether the enhanced effects of LIPUS are dependent on the timing and duration of LIPUS treatment in this model to determine the target reaction of LIPUS in rhBMP-2 induced bone formation.

METHODS: In an IACUC approved study, the left femurs of 120 male SD rats were internally stabilized with a custom fabricated HDPE fixator and SS screws. A 5-mm mid-diaphyseal segment of bone was excised and replaced with rhBMP-2 (1.2µg) loaded on absorbable collagen sponges (ACS). The study period was 4 weeks and this interval was divided into four equal phases (Ph-1, Ph-2, Ph-3 and Ph-4; N=30) of LIPUS treatment. 15 animals from each group received daily LIPUS for 20 min. The remaining 15 animals received sham LIPUS exposure. All animals were sacrificed at 4 weeks. All femurs were analyzed with radiographs and μCT (BV/TV) and further processed for either torsion tests (external moment, 3°/sec) or histology. Results were also compared to intact controls and historical data from animals receiving rhBMP-2 (0µg or 1.2µg) + LIPUS treatment throughout (Th) the 4 weeks. Data was analyzed using multivariate ANOVA or paired t-test.

RESULTS:

μCT: A decreasing trend was observed in the BV/TV with the sham treatments at different phases which was rescued by the LIPUS treatment (no significant change between phases of LIPUS treatment) (Fig 1). LIPUS at Ph-2 showed a drop in BV/TV versus its respective sham control whereas LIPUS at Ph-3 showed a significant increase in the BV/TV versus its respective sham control. LIPUS at Ph-2, Ph-3 and Ph-4 showed significant increase in BV/TV as compared to sham Th. Histology: There was an incomplete union with a prominent residue of the ACS in the defect region surrounded by cartilaginous tissue in all the sham treated groups. The residual ACS was not seen in the LIPUS groups (Fig 2). With LIPUS (Ph-2) the cartilage areas had reduced. With LIPUS Ph-3 there was minimal cartilage with new bone surfaces lined with osteoblasts. LIPUS Ph-4 was the only group which showed active osteoblast and osteoclast present in all surfaces of the hard callus.

Bio/mechanics: Similar to BV/TV data, a decreasing trend was observed in the peak torsion with sham treatments at different phases. LIPUS rescued this drop in Ph-3 and Ph-4 (no significant changes between phases of LIPUS treatment) (Fig 3). There was no effect of LIPUS treatment in the Ph-1. With LIPUS in Ph-2 the peak torsion decreased (P=0.053). LIPUS at Ph-3 significantly increased the torsion strength as compared to sham Th.

DISCUSSION: By dividing the LIPUS treatment regime into four phases we sought to investigate if the enhancing effects of LIPUS were due to its role in the inflammatory and angiogenic (Ph-1), chondrogenic (Ph-2), osteogenic (Ph-3) or remodeling (Ph-4) phase(s) of healing. Our findings suggest that there is no direct effect of LIPUS on bone formation in the initial inflammatory phase or angiogenic phase (Ph-1). Nevertheless, we did observe that treatment with LIPUS throughout the healing period showed increased newly forming and matured blood vessels in the defect. The use of LIPUS in the chondrogenic phase (Ph-2) might be influencing an unwavering osteogenic differentiation and activity in the early times thus providing an early onset to mineralization, and appearing less efficient due to the lesser BV/TV and mechanical strength. The use of LIPUS in the osteogenic phase (Ph-3) was most beneficial as it seems to enhance the bone formation and mechanical strength. The use of LIPUS in the remodeling phase (Ph-4) is the only group to show lamellar bone. In conclusion, the best phase to use LIPUS to enhance rhBMP-2 induced bone formation is the osteogenic phase while the use in the final remodeling phase is also beneficial due to its potential ability to promote callus maturation.

SIGNIFICANCE: LIPUS can be used in combination with a clinically safe dose of rhBMP-2 in the osteogenic and/or remodeling phase to enhance/accelerate the healing process.

ACKNOWLEDGEMENTS: NIH-NIAMS and Orthopedic Trauma Association. David Karwo & Julie Brown (technical help), Dr. Vincent Wang (mechanical testing), Smith & Nephew, Inc. (material donation).