Effect of Combined Treatment with Recombinant Human Bone Morphogenetic Protein-2 and Low-Intensity Pulsed Ultrasound in Ectopic Bone Formation

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Introduction: Biochemical and mechanical signals play crucial roles in bone growth and repair. Exogenous application of growth factors and low intensity pulsed ultrasound (LIPUS) are effective in enhancing/accelerating bone healing in a number of animal models. However, the combined use of these two modalities in neo-osteogenesis has not been reported. To address this deficiency, we investigated the effect of combined treatment of recombinant human bone morphogenetic protein-2 (rhBMP-2) and LIPUS in an ectopic model of bone formation in the rat. We hypothesized that the combined application of rhBMP-2 and LIPUS will cause an additive or synergistic effect compared to either treatment alone.

Materials and Methods: 16 Long Evans rats (male, age 11 weeks) each received 4 absorbable collagen sponges (ACS) loaded with 0 μg (buffer only), 1μg, 2.5μg, or 5μg of rhBMP-2. The loaded ACS were implanted subcutaneously on the back so that LIPUS transducers could be placed directly on the implant sites. Half the animals were treated daily with LIPUS(1.5 MHz, repetition rate 1kHz, 200 μs burst, 30 mW/cm2SATA). The other half received sham LIPUS treatment. LIPUS and sham groups were sacrificed at 2 and 4 weeks post-surgery. Tissues from the implantation sites were analysed by micro-computed tomography (μCT) to determine bone volume. Toluidine Blue and von Kossa staining was performed on plastic embedded sections to assess tissue morphology.

Results: At 4 weeks, rhBMP-2 alone showed dose dependent bone formation (bone volume) in ACS (p=0.004). A similar pattern, with elevated response, was observed when LIPUS was applied in addition to rhBMP-2. LIPUS enhancement of bone formation was highest in the 1μg rhBMP-2 (16.8 fold, p=0.02) followed by 8.6-fold and 2.9-fold increases in the 2.5μg (p=0.28) and 5μg (p=0.07) groups, respectively. No bone was detected where rhBMP-2 was absent (buffer only) even when LIPUS was applied. No new bone formation was observed at the 2 week time point(data not shown). von Kossa staining of mineralized tissue confirmed the μCT findings (Figure 2). Toluidine Blue staining revealed normal cellular events of bone formation with newly formed osteoid lined with active osteoblasts.

Discussion: The novel finding of this study is that LIPUS treatment enhanced rhBMP-2-induced bone formation in vivo. Our results that rhBMP-2 initiated new bone formation at ectopic sites in a dose-dependent manner is consistent with previous reports. The LIPUS-related enhancement of bone formation, although observed to some degree at all three doses of rhBMP-2, was statistically significant only at the low dose, possibly because the rate of bone formation at higher doses of rhBMP-2 was nearly maximized, thereby dampening the responsiveness to the additional stimuli provided by LIPUS.

The mechanism by which LIPUS enhances rhBMP-2-induced bone formation is not known. Both LIPUS and rhBMP-2 are known to act on different phases of osteogenesis and together they may act in a sequential manner on selective phases, thus leading to enhanced bone formation. In our previous work, we have reported that combined treatment with LIPUS and rhBMP-2 had no additive effect on gene expression in rat bone marrow stromal cells in vitro [1]. Therefore, it is possible that LIPUS acts on a population of cells in vivo that are not represented in in vitro culture systems or that LIPUS makes cells more responsive to local biological signals.

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