Combination Ex Vivo Regional Gene Therapy with Sonic Hedgehog and Bone Morphogenetic Protein-2 for Critical Size Bone Defects

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INTRODUCTION: Critical size bone defects pose significant challenges for orthopaedic surgeons. Regional gene therapy using lentiviral vector (LV) Bone Morphogenetic Protein 2 (BMP-2) transduced Adipose Derived Stem Cells (ADSCs) have been used in preclinical models to heal critical size femoral defects. Sonic hedgehog (Shh) protein has recently been investigated for its role in osteogenesis, with studies suggesting a synergistic effect with BMP-2. The purpose of this study was to evaluate if combination gene therapy with rat ADSCs transduced with LV-BMP-2/LV-Shh can heal a rat critical size femoral defect with a reduced cell dose of BMP-2.

METHODS:

Seventy-one rats underwent femoral defect surgery and were treated with either 5 million (5 M) or 3 million (3 M) LV-transduced ADSCs. Animals were randomly assigned to the following groups: (I) LV-BMP-2/LV-Shh 5 M, (II) LV-BMP-2/LV-Shh 3 M, (III) LV-BMP-2 5 M, (IV) LV-BMP-2 3 M, (V) LV-Shh 5 M, (VI) non-transduced 5 M, or (VII) β-TCP carrier only. Femurs were harvested 12-weeks post-operatively and underwent biomechanical testing, radiographs, micro-CT, and histomorphometry assessment.

RESULTS:

At 12 weeks postoperatively, the majority of femurs in Group I (9/14; 64%), II (10/14; 71%), III (9/14; 64%), and IV (7/14; 50%) had complete radiographic healing. Conversely, no femurs in Groups VI and VII healed or achieved more than 50% cortical restoration. There was no significant difference in stiffness, total energy, or maximum torque to failure between Groups I-IV. Radiographic analysis found that Group I and II had non-inferior healing compared to Groups III and IV. Group I had significantly greater bone volume than group III on micro-CT analysis.

DISCUSSION AND CONCLUSION:

We found LV-BMP-2/LV-Shh 5 M had equivalent stiffness, torque, and total energy to failure as LV-BMP-2 5 M alone. Furthermore, combination therapy with LV-BMP-2/LV-Shh 3 M also demonstrated non-inferiority to LV-BMP-2 5 M alone on biomechanical testing. This represents a 40% reduction in cell dose required to achieve equivalence for our primary outcome measure when compared to previous studies testing LV-BMP-2 transduced ADSCs in this model. Our findings support the hypothesis that BMP-2 and Shh can act synergistically to heal a critical sized defect and reduce BMP-2 dosing requirements. A reduction in cell dose has important implications for clinical translation of gene therapy by minimizing resources required for cell harvest, isolation, culture, and transduction.

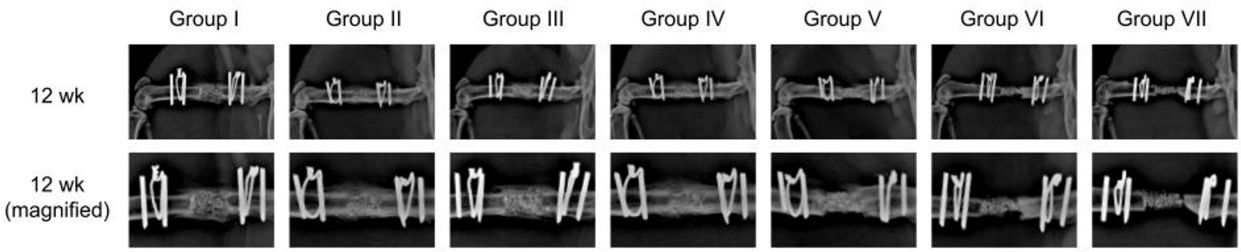

Figure 1. Radiographic imaging at 12 weeks. Radiographs were obtained to evaluate the healing of study groups