Systemic Administration of Sclerostin Antibody Enhances BMP Induced Bone Repair

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Disclosures:
J.R. Lieberman: 3B; Consultant- Depuy. 4; Hip Innovation Technology. 5; Amgen, Inc..

Introduction:
Recombinant human bone morphogenic protein-2 (BMP2) is a potent osteoinductive agent, but its efficacy in clinical trials has been mixed. Sclerostin is a negative regulator of osteoblast development and bone formation and may act by inhibiting the BMP and canonical Wnt signaling pathways. A number of pre-clinical studies have demonstrated that the inhibition of sclerostin with sclerostin antibody (Scl-Ab) can enhance bone formation and fracture healing. However, in a rat segmental defect model, although Scl-Ab treatment enhanced bone formation within the defect, the biologic effect of the SclAb was not sufficient to consistently heal the defect. In this model, rhBMP2 consistently promoted healing across the defect, though full recovery of intact bone strength was not achieved. Our hypothesis was that combined treatment of an osteoinductive agent such as local rhBMP2 and systemic administration of Scl-Ab would lead to enhanced bone repair compared to rhBMP2 alone in a rat femoral critical sized defect model.

Methods:
Our protocol was approved by our Institutional Animal Care and Use Committee. A rigidly fixed 6mm critical femoral defect was created in fifty-five male Lewis rats. A defect was made in a single hindlimb of each animal. The animals were randomly assigned into a group at the time of surgery. Group 1 (n=15) received only a collagen sponge with saline in the defect at the time of surgery. Group 2 (n=20) received BMP2 on a collagen sponge in the defect at the time of surgery (10ug BMP2) and saline injections twice per week (volume matched to Scl-Ab injections). Group 3(n=20) received BMP2 on a collagen sponge in the defect at the time of surgery (10ug BMP2) and Scl-Ab injections twice per week (25mg/kg subcutaneous). All rats were sacrificed 12 weeks after femoral defect surgery. All samples were evaluated with plain x-ray and microCT. Five samples from each group were evaluated by histologic and histomorphometric analysis. The remaining samples from Scl-Ab+BMP2 (n=12) and BMP2 alone (n=15) underwent torsional biomechanical testing calculating maximum torque to failure, energy to failure and torsional stiffness. This was compared to a group of nonoperative intact femurs from control animals (n=15). All data are given as means ± standard error (SE). Samples were compared for differences with a one-way analysis of variance (ANOVA) and a Bonferroni post-hoc analysis. A p-value < 0.05 was considered a significant difference.

Results:
By x-ray and microCT, none of the control samples healed (0/15), while all of the BMP2 only (20/20) and Scl-Ab+BMP2 (20/20) samples healed (Figure 1). MicroCT volumetric analysis revealed Scl-Ab+BMP2 had significantly higher bone volume (BV, Figure 2) and total volume (TV) compared to BMP2 alone (p<0.01) and control defect (p<0.01). In addition, Scl-Ab+BMP2 had a significantly higher bone volume fraction (BV/TV) compared to control (p<0.01), but was not significantly different than BMP2 alone (p=0.537). Mid-defect axial histomorphometry was consistent with the microCT results showing Scl-Ab+BMP2 had a significantly greater bone area (BA) than BMP2 alone (0.252±0.046 vs. 0.143±0.019; p=0.029) and control defect (0.098±0.083; p=0.003). Analysis of tissue area (TA) showed no difference among the groups while the bone area fraction (BA/TA) was significantly greater in Scl-Ab+BMP2 versus control defect (46.76±5.91 vs. 19.47±11.53; p<0.001) and in BMP2 alone versus control defect (36.15±4.99 vs. 19.47±11.53; p=0.019). The difference in bone area fraction between Scl-Ab+BMP2 and BMP2 alone was not significant (p=0.175) (figure 3). Biomechanical testing comparing experimental samples with intact control femurs revealed Scl-Ab+BMP2 had a significantly higher maximum torque to failure than BMP2 alone (p<0.001) and intact control (p<0.001). In addition, the intact control group had a significantly higher torque to failure than BMP2 alone (p=0.002) (figure 4). Torsional stiffness was significantly greater in Scl-Ab+BMP2 versus BMP2 alone (0.064±0.242 N-m/deg vs. 0.046±0.021N-m/deg; p=0.047) and intact control (0.033±0.01 N-m/deg; p<0.001) while the difference between BMP2 alone and control was not statistically significant (p=0.200). Energy to failure was significantly greater in Scl-Ab+BMP2 compared to BMP2 alone (8.30±5.93 N-m*deg vs. 2.86±2.01 N-m*deg; p=0.001). While mean energy to failure was greater in Scl-Ab+BMP2 versus intact control (5.80±1.79 N-m*deg; p=0.228) and greater in control compared to BMP2 alone (p=0.086), these were not
Discussion:
The results of this study demonstrate that systemic administration of Scl-Ab combined with local delivery of BMP2 is superior to BMP2 alone for healing critical sized rat femoral defects. MicroCT and biomechanical testing show that in rats treated with Scl-Ab+BMP2 more bone is formed in a larger callus that is both stronger and more rigid than in BMP2 alone and normal nonoperative femurs. Clinical and preclinical studies describe the resorptive effect of osteoinductive agents such as BMP2. While BMP2 is a potent activator of osteoprogenitors, it is coupled to osteoclastic mediated bone resorption. Evidence suggests that Scl-Ab mediated bone formation is uncoupled and does not cause a resorptive response. Although Scl-Ab was unable to consistently heal critical sized defects alone, together with an osteoinductive agent (rhBMP-2) it improved the bone volume and strength of the healing response compared to either agent alone.

Significance:
Despite advances in surgical techniques, delayed fracture healing and fracture nonunion are difficult to treat. Combining an osteoinductive agent with a systemic anabolic agent may be a new paradigm in the treatment of fracture non-unions. This study demonstrates that systemic Scl-Ab treatment combined with local rhBMP2 is better than rhBMP2 alone in treating critical sized rat femoral defects, which suggests that this regimen may have potential as a therapeutic regimen in humans.

Acknowledgments:

References:

Figure 1

X-rays of rat femurs with a 6mm defect rigidly fixed with a polyethylene plate, threaded pins and circlage wires. After 12 weeks, no control group defects healed (A), all BMP2 only (B) and Scl-Ab+BMP2 (C) defects healed.

Figure 2

MicroCT of rat femurs 12 weeks postoperatively with volumetric rendering (A-C) and measured bone volume (D). Scl-Ab+BMP2 made the most bone (C, D). Data expressed as mean ± SE, *P<0.01 versus control defect, †P<0.01 versus BMP2 alone.
Figure 3

Histology obtained from mid-defect axial sections (D-F). Scl-Ab+BMP2 (C,F) made significantly more bone compared to BMP2 alone (B,E) and control defect (A,D) as measured by bone volume histomorphometry.

Figure 4

Max Torque to Failure (N-m)

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<th>N=15</th>
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<th>N=12</th>
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<tr>
<td>Intact femur</td>
<td>0.592</td>
<td>0.455</td>
<td>0.913</td>
</tr>
<tr>
<td>Control</td>
<td>±0.026</td>
<td>±0.061</td>
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Torsional biomechanical testing shows Scl-Ab+BMP2 had a greater torque to failure than both BMP2 alone and intact control femur. Control femur had a significantly higher torque to failure than BMP2 alone. *P<0.001 vs BMP2 alone and vs control, †P<0.002 vs BMP2 alone

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