The Effect of Systemic Administration of Sclerostin Antibodies in A Mouse Model of Distraction Osteogenesis

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Introduction: Distraction osteogenesis (DO) is a surgical technique widely used to treat limb length discrepancy, limb deformities, long bone nonunion and bone loss(1). One limitation of this technique is the long time that the external fixator is left in place until the bone is completely consolidated. This might lead to significant morbidities in terms of persistent pain, increase risk of pin tracts infection and negative psychological impact on children and their families(1). Sclerostin is a secreted glycoprotein that interacts with the lipoprotein receptor-related protein 5 (LRP5) and inhibits the intracellular Wnt signaling pathway, leading to decreased bone formation activity by osteoblasts. When Sclerostin is inactivated, bone formation is therefore stimulated. We hypothesized that the systemic administration of sclerostin antibodies (Scl-Ab) can accelerate bone regeneration in a mouse model of DO.

Methods: A total of 110 mice were randomized to saline versus Scl-Ab injection groups. After DO surgery in the right tibiae, mice were injected intraveously once weekly with Scl-Ab (100mg/kg) versus saline(0.1 ml). Mice were sacrificed at four time points, day 11(mid-distraction phase), day 17(end of distraction), day 34(mid-consolidation) and day 51 (end of consolidation). Radiographic (Faxitron), microstructural (μCT) and qualitative histological analysis was performed for the lengthened tibiae at all time points. In addition, biomechanical testing was performed at day 34 and 51.

Results:
Of 110 mice, 95 were included in the analysis of this study (The schematic depiction of DO procedure and final sample allocation across time points and tests are summarized in image.1). Micro CT results showed an increase of bone volume in the Scl-Ab group when compared with the control group at day 11 (P=0.009). A trend toward increase bone volume was observed at day 17, day 34 and day 51 (P>0.05) [μCT (Top panel) and radiological images (bottom panel) of lengthened limbs collected from saline and Scl-Ab groups at 4 time points in image.2]. The qualitative histology showed predominat presence of chondrocyte and fibrocartilage in Scl-Ab group when compared with saline group at day 11(Image.3: histological images of distracted limbs of saline and Scl-Ab groups at 11 days). Radiographic bone fill scores were higher in Scl-Ab group when compared to saline group (Table-1). Biomechanical analysis revealed trend toward higher values of stiffness, ultimate force and work to ultimate point in Scl-Ab groups when compared to saline groups at day 34 and day 51 (P>0.05).

Discussion: To the best of our knowledge, this is the first report on the effects of Scl-Ab on bone regeneration during DO. The μCT results showed increased bone volume at day11 in the Scl-Ab group when compared with the control group (P=0.009). Additionally, the qualitative histological analysis showed predominat presences of chondrocytes and fibrocartilage at day 11 in Scl-Ab specimens when compared to the control specimens. These findings postulate that Scl-Ab had its maximum effect during the distraction phase of DO. This corroborates the evidence of our previous report in which we found that the highest expression of Wnt positive regulators was at the distraction phase while it decreased during the consolidation phase(2). Our results have also shown that the biomechanical results of work to ultimate point were higher in Scl-Ab group at day 34 when compared to the control group at day 51. Additionally, the ultimate force values in Scl-Ab group at day 34 were approximately equivalent to the results of day 51 in the control group. This could suggest that the effect of Scl-Ab on the bone quality at mid-consolidation phase (day 34) would resemble the bone quality of the control group at the end of consolidation(day 51). This is promising as such fact might indicate that the removal of external fixator is possible in a shorter period with sufficient bone quality to stand for the weight bearing loads.

In bone repair and implant fixation models, there exist controversy whether Scl-Ab has its maximal effect during the repair phase (early after injury) or remodeling phase (late after injury)(3, 4). While based on our results and previous immunohistochemical study of Wnt pathway during DO(2), It appears that the maximum benefit of the systemic administration of Scl-Ab would be during the distraction phase. This might suggests that the Scl-Ab injections are not required during the consolidation phase of DO. In fact, this would minimize any potential side effects of Scl-Ab secondary to its prolonged systemic administration.

In conclusion, the systemic delivery of Scl-Ab led to acceleration of bone regeneration during DO although that trend did not reach statistical significance in most parameters. Its effect was mostly during the distraction phase. This indicates the potential clinical utility of systemic administration of Scl-Ab in DO to reduce the treatment period of the external fixator.

Significance: Our data suggest the potential utility of Scl-Ab in clinical situations to reduce the treatment period with an external
fixator during DO procedures.

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**References:**


![Diagram](image)

2010/05/26.

![Images](image)

**Day 11 “Saline group”**  **Day 11 “Scl-Ab group”**

Average bone fill score as graded by 3 blinded reviewers.
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<thead>
<tr>
<th>Time point</th>
<th>Scl-Ab group</th>
<th>Saline group</th>
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<td>Day 11</td>
<td>0.7</td>
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<td>Day 34</td>
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<td>Day 51</td>
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