Enhanced Healing During Distraction Osteogenesis in Rats with Sclerostin Antibody

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Introduction
Sclerostin (Scl) is a negative regulator of osteoblast differentiation and bone formation, via antagonism of the Wnt/β-catenin pathway and/or BMP activity. The expression pattern of this protein is thought to be highly restricted to osteocytes, localizing its effects to skeletal tissue. A sclerostin antibody (Scl-Ab) has recently been reported to increase bone formation in ovariectomized rats and enhance fracture healing in mouse and rat models. A first in human study shows dose-dependent increases in bone formation markers and increases in BMD. Distraction osteogenesis is now a standard orthopaedic procedure used in limb lengthening and limb reconstruction surgery. Complications of this technique include disuse osteopenia and poor anabolic response, both of which would benefit from pro-anabolic therapy. We sought to examine the effects of Scl-Ab in a rat model of distraction osteogenesis.

Hypothesis
Scl-Ab treatment will enhance healing in a rat distraction osteogenesis model via enhanced stimulation of bone formation.

Methods
An osteotomy in the femur was stabilized with an external fixation lengthening device in 43 male Sprague Dawley rats. After a week of latency, the gap was distracted at a rate of 0.25mm daily for 44 days to a total of 7mm. Saline or Scl-Ab (Scl-AbIII) was administered twice weekly throughout the distraction period and up to 4 or 6 weeks post commencement of distraction (Figure 1). Three groups were examined, Saline, Continuous Scl-Ab (C Scl-Ab) and Delayed Scl-Ab (D Scl-Ab, post distraction only). Samples were radiographed (Faxitron MX-20) to assess union rates. DXA scans of the regenerate and proximal intact bone were performed on all samples at 2, 4 and 6-week time points and all harvest samples were scanned using Micro CT (µCT) for 3D analysis of bone architecture. Samples were then processed for undecalcified histology to measure 2D bone architecture and bone formation parameters using double fluorochrome labels.

Results
Radiographs demonstrated a trend for enhanced union rates with Scl-Ab treatment at 6 weeks with the following unions/N for each group Saline (1/5) D Scl-Ab (3/7) and C Scl-Ab (4/8) (Figure 2).

Discussion
Scl-Ab treatment enhanced the amount of bone formed in this rat distraction model as assessed by DXA, µCT and histology. Both continuous and delayed treatment showed significant effects on regenerate formation and consolidation over controls.

Histological analysis at 6 weeks confirmed uCT outcomes with 130-140% increases in BMD in the C Scl-Ab group compared to Saline (p<0.01). This was due to an increase in BMC of 117% in this group at 2 weeks (p<0.01). At the 6-week time point, regenerate bone area was increased 23% in both D Scl-Ab (p<0.05) and C Scl-Ab (p<0.01) compared to Saline. Further, C Scl-Ab treatment increased BMC proximal to the regenerate compared to Saline at 2, 4 and 6 weeks, (p<0.05)

Micro CT scans of the regenerate region revealed an 89% increase in bone volume with C Scl-Ab treatment compared to Saline at 6 weeks (p<0.05). Bone volume ratio (BV/TV) was increased 77% in C-Scl Ab and 82% in D-Scl Ab compared to Saline treatment at this time point (p<0.05, figure 3)

Figure 3. 3D images of representative samples from each group at 6 weeks generated from uCT scans

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Figure 4. Representative images of calcein labels showing larger interlabel distance in C-Scl Ab and D-Scl Ab treated samples.

Conclusion
Scl-Ab treatment increased bone formation in this model of distraction osteogenesis resulting in a larger callus. We expect further studies to reveal increases in mechanical strength of the regenerate. As such Scl-Ab is a candidate for clinical development to accelerate regenerate formation and consolidation in distraction osteogenesis, which could shorten time spent in the fixator and decrease disuse osteopenia-related complications such as re-fracture.

References
3. Pahdi et al JBMR Epub

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