SCLEROSTIN ANTIBODY ENHANCES BONE REPAIR IN A RAT FEMORAL DEFECT MODEL

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INTRODUCTION:
The Wnt signaling pathway plays an important role in regulating bone formation and Wnt inhibitors are targets of pharmacotherapy to enhance bone formation. Sclerostin is a secreted glycoprotein that is expressed by mature osteocytes and negatively regulates the canonical Wnt pathway by binding to the LRP5/6 receptor. Systemic administration of a sclerostin-neutralizing monoclonal antibody (Scl Ab) has been shown to enhance bone formation, bone mass and bone strength in aged ovariectomized rats1. In addition, Scl Ab has been shown to increase callus density and strength in murine and primate osteotomy healing models2. A previous study in a 6 mm critical size defect model in Lewis rats demonstrated that radiographic healing occurred in 20% of Scl Ab treated femurs3. In order to further evaluate the potential of Scl Ab to improve healing in a non-critical size defect model, we tested Scl Ab in a 3 mm femoral diaphyseal defect in young male outbred rats.

MATERIALS AND METHODS:
3 mm full thickness defects were created in the mid-shaft of the left femur of 12-weeks-old male Sprague Dawley rats and were fixed internally as previously reported4. Two groups of rats received subcutaneous injections of Vehicle (PBS) or 25 mg/kg Scl Ab twice weekly for 12 weeks immediately after surgery, while a third group received 25 mg/kg Scl Ab for 10 weeks after a 2-week delay (D-Scl Ab; n = 15 per group). After termination at 12 weeks, radiographs of the operated femurs were assessed by 3 independent observers to score the defect healing from 0 to 5 based on a previously published protocol4. All of the operated femurs and 5 randomly selected contralateral femurs were subjected to micro-CT analysis. Data were expressed as Mean ± SE, and group comparisons were made using an ANOVA + Tukey’s post test, with significance at p<0.05.

RESULTS:
Figure 1 illustrates the progression of gap defect healing in each treatment group after 12 weeks, by ex-vivo radiographic and micro-CT images. After 12 weeks, radiographic healing (score ≥ 4) was observed in 5/15 in the D-Scl Ab group and 2/15 in both the Scl Ab and Vehicle groups. As assessed by micro-CT, defect union occurred in 3/15 in the D-Scl Ab group, 2/15 in the Scl Ab group, and 1/15 in the Vehicle group. The mean radiographic score for the D-Scl Ab group, but not the Scl Ab group, was significantly higher than in the Vehicle group (Figure 2). The micro-CT analysis demonstrated a significant 65% increase in bone volume fraction (BV/TV) in the gap defects of the D-Scl Ab group compared to Vehicle, while the Scl Ab increased BV/TV by a non-significant 35% (Figure 2). In the contralateral femurs, Scl Ab and D-Scl Ab resulted in similar increases in distal femur BV/TV, distal femur trabecular (Tb) thickness, and femoral shaft cortical (Ct) thickness relative to Vehicle (Table 2).

DISCUSSION:
Fracture union in this 3 mm gap defect model was expected to occur in the majority of rats in the Vehicle group by 12 weeks as determined in a pilot study in Lewis rats. The micro-CT results indicated that fracture union occurred in less than 7% of Vehicle-treated rats, thus the hypothesis regarding the effects of Scl Ab in a non-critical size defect could not be fully tested. However, similar to our previous report in a 6 mm critical size defect model in Lewis rats5, Scl Ab treatment did result in increased bone volume in the 3 mm defect in Sprague Dawley rats after 12 weeks. In the current study, the improvements in radiographic scoring were significantly greater with a 2-week delay prior to treatment compared to Vehicle or continuous Scl-Ab dosing. The reason for these observations is unclear, but could reflect the variability in surgical technique, variability in baseline bone size and density, or a less optimal effect of early sclerostin inhibition. In the previous 6 mm defect study, 2 weeks of Scl Ab treatment either immediately after or 2 weeks after fracture resulted in similar modest improvements in radiographic scoring and BV/TV, suggesting that early Scl Ab treatment did have a positive effect on healing. The 6 mm defect study also demonstrated that continuous Scl Ab resulted in improvements in both radiographic score and defect BV/TV, unlike the current study. The variable response to Scl Ab treatment in these studies may be related to the different healing responses in these 2 rat strains.

REFERENCES:
1Li et al. 2009 JBM 24:578-88
2Ominsky et al. 2010 Osteoporos Int 21: S29
3Virk et al. 2009 Trans ORS 34: 607
4Lieberman et al. 1999 JBJS Am 81:905-17