Systemic Administration of a Sclerostin Neutralizing Antibody Enhances Fracture Healing by Stimulating Bone Formation in the Fractured Gap in a Nonhuman Primate Fibular Osteotomy Model

+Ominsky MS; Samafdam R; Jolette J; Vlasserus P; Smith SY; Paszy C; Simonet WS; Ke HZ

1 Amgen, Thousand Oaks, CA, USA, 2 Charles River Laboratories Preclinical Services Montreal, Inc., Montreal, PQ, Canada mominsky@amgen.com

INTRODUCTION

Beta-catenin signaling has been reported to play an important role in fracture healing2. Sclerostin, a protein secreted by osteocytes, is a negative regulator of osteoblast differentiation/function3 via inhibition of Wnt/beta-catenin signaling4. Inhibition of sclerostin via monoclonal antibodies (Scl-Ab) has been shown to increase systemic bone formation in rats, primates, and post-menopausal women4-6. Scl-Ab also increased fracture strength in models of fracture healing in mice and rats7. Nonhuman primates provide an excellent model of fracture healing in humans due to similarities in anatomy, cortical bone remodeling, and time course of healing. Scl-Ab was previously shown to increase fracture strength in a nonhuman primate fibular osteotomy model8. The objective of the current study was to investigate the effects of systemic administration of Scl-Ab on histologic bone formation and healing in the nonhuman primate fibular osteotomy model.

METHODS

All animal activities were approved by the Charles River Montreal Animal Care Committee and performed in an AAALAC-accredited facility. Forty-four male cynomolgus monkeys (cynos, aged 4-5 years) underwent bilateral fibular osteotomies with intramedullary (IM) fixation as previously described. After surgery, the animals were injected subcutaneously with vehicle (Veh) or sclerostin monoclonal antibody (Scl-Ab, 25 mg/kg) biweekly for 10 weeks. Blood was collected biweekly and DXA BMD was assessed at baseline, 6, and 10 weeks. The IM pin was removed in one fibula per monkey, which was scanned ex-vivo by pQCT (XCT Research SA) and tested in torsion to failure (MTS). Thresholds of 0.400 and 1.090 cm² were applied to assess the total and hard (mature) callus, respectively.

Semi-quantitative scoring (SQS: 5-point scale) was performed on ground, plastic longitudinal fibular sections (n=10/group) to assess the callus size, extent of delayed/non-union, extent of cartilage, extent of bone in the gap, and cortical porosity. Histomorphometry of the fracture site quantified the callus area and its composition (bone, cartilage) as well as the gap area and composition (bone, fibrous/granulation tissue). Dynamic histomorphometry was performed at the 2nd lumbar vertebra (L2) and femur neck (FN) (n=10/group). Bone formation rate (BFR/BS) was assessed separately for the first set of tetracycline labels (days 14 and 24; BFR-1) and second set of calcein labels (days 56 and 66, BFR-2).

Animals with notably bent pins and/or obvious displacement of bone ends at the osteotomy site were excluded from analysis. In addition, 4 animals which developed drug clearing antibody during the study period were also excluded from analysis.

RESULTS

As reported previously, Scl-Ab resulted in significant increases in serum bone formation markers. DXA BMD at the lumbar spine, hip, and distal radius, and lumbar vertebral strength. At the fracture site, Scl-Ab treatment resulted in a significant increase in total callus bone mineral content (BMC), as well as hard callus area and BMC (all p<0.05 vs Veh). The Scl-Ab mediated improvements in callosus maturity were associated with a 48% greater mean torsional stiffness compared to vehicle controls (p<0.05), while maximum torque was increased by a non-significant 32% (n=12/group).

Fracture histology: Scl-Ab treated fibulae demonstrated a lower incidence of delayed union compared to Veh (10% vs 67%), with a significant decrease in scoring (Table 1). Delays in fracture union corresponded with group differences in both the callus and the gap spanning the osteotomy as demonstrated in Figure 1. The calluses in the Scl-Ab group were less cartilaginous than both SQS (Table 1) and histomorphometry (-80%, Table 2) compared to Veh. The osteotomy gaps in the Scl-Ab group had less fibrous/granulation tissue area (+94%) and were more filled with bone by both SQS and histomorphometry (+37%). Gap area was itself significantly lower in the Scl-Ab group (+59%), perhaps as a consequence of greater resorption at the osteotomy site in Veh controls. No group differences were found in cortical porosity (mean SQS = 2.5 for both groups).

DISCUSSION

Inhibition of sclerostin by systemic treatment with Scl-Ab improved fracture healing by increasing bone formation between fractured bone ends and increasing remodeling of cartilage to bone in the external callus in male nonhuman primates. In addition, systemic treatment with Scl-Ab significantly increased bone formation and bone mass in other non-fracture skeletal sites such as lumbar vertebral and femoral neck. Such an efficacy profile would provide important benefits in patients who have fractures to augment their healing while reducing future risk of fracture at other skeletal sites.

REFERENCES

8. Ominsky MS et al. J Bone Miner Res. 2009;24(suppl. 1):1290