Sost Antibody Treatment Improves Fracture Healing in Type 1 Diabetes

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Introduction: Type 1 diabetes mellitus (T1DM) patients suffer from low bone mass, increased fracture risk, and impaired fracture healing due to reduced osteoblast activity. Available treatments can improve bone mineral density (BMD) in T1DM [1] [2], however, there is no specific treatment that can adequately restore the fracture healing phenotype. Since over 8% of the US population suffers from T1DM, a great need exists to explore new opportunities for treating orthopedic complications. Sclerostin (Sost), a Wnt antagonist, is secreted by osteocytes and inhibits bone formation. Sost knockout mice (Sost KO) have 3X higher BMD than normal and enhanced fracture healing due to increased Wnt signaling [3]. Sost antibodies (SostAb) have been shown to enhance bone healing in animal models by increasing bone formation and mass [4] due to enhanced osteoblast function, determined by increased osteocalcin protein (a bone formation biochemical marker) and decreased osteoclast number/total area [5]. SostAb treatment in type 2 diabetic rats has been shown to improve bone mass and strength [5], however this effect has not yet been examined in T1DM. We have examined the effects of SostAb treatment on fracture repair in Streptozotocin (STZ)-induced T1DM mice. Our preliminary results suggest that SostAb helps heal problematic fractures in a T1DM mouse model, by directly enhancing osteoblast function.

Methods: To induce T1DM, STZ was administered (50mg/kg) for 5 days to 6 weeks old C57Bl6/J (WT) mice. Blood glucose readings of >300 mg/dL confirmed diabetic status of STZ treated mice. Mid-femoral fractures were generated in a closed Einohorn model at 8 weeks of age, and SostAb (25 mg/kg) was administered twice weekly up to 21 days post-fracture (sham-treated WT, sham-treated STZ, WT+SostAb, and STZ+SostAb groups) and discontinued for three weeks until 42 day post-fracture. At 21 days (d21) and 42 days (d42) post-fracture, bones were dissected and processed for micro-CT or histology and immunohistochemistry (IHC). All animal work was performed under an IACUC-approved Animal Use Protocol at an AAALAC-accredited facility.

Results: Micro-CT analysis at d21 post fracture showed STZ calluses had a significant decrease (~50%; p-value 0.0155) in total volume in comparison to sham treated WT, consistent with a delayed healing phenotype. Histologically, less bridging and woven bone was observed in STZ calluses compared to WT, suggesting that STZ calluses are smaller with less mineralized bone, consistent with published reports of poor osteogenesis [6] [7]. Bone volume/total volume (BV/TV) ratios at d21 showed that STZ+SostAb calluses had a significant increase in mineralized bone relative to both sham treated WT and STZ groups (p-values < 0.0008) (Fig. 1A). Histologically stained paraffin sections of STZ+SostAb calluses at d21 showed enhanced bone formation compared to WT and STZ calluses, confirming the positive effects of SostAb treatment on T1DM fracture healing. SP7/Osterix, an osteoblast marker, was elevated on d21 in...
STZ calluses, suggesting that osteoblast differentiation is not altered in STZ mice. However, Collagen I, a matrix protein secreted by osteoblasts was reduced at d21 in STZ calluses, while WT+SostAb and STZ+SostAb calluses showed increased signal. Micro-CT analysis at d42 revealed a significant increase in BV/TV (p-value < 0.01) of STZ+SostAb calluses compared to sham treated STZ or WT, suggesting SostAb improves T1DM fracture healing by enhancing bone formation (Fig.1B). At d42, histological stained paraffin sections of STZ+SostAb calluses continued to show enhanced bone formation at the fracture site compared to STZ calluses, even after discontinuation of SostAb treatment at 21 days post-fracture. **Discussion:** SostAb treatment in STZ-induced T1DM mice promotes fracture repair and rescues the reduced osteogenesis caused by T1DM. Since SostAb up-regulates Wnt signaling, our results suggest that the enhanced osteoblast activity observed is independent of glucose metabolic pathways. SostAb has a potent effect on bone formation which does not seem to reverse after the treatment is interrupted, suggesting that SostAb could potentially correct the fracture repair defects associated with T1DM and could have a lasting positive effect on BMD. While osteoblasts differentiation seems unaltered in STZ-induced T1DM, we find that osteoblasts synthesize less Collagen 1 matrix protein. We are further investigating whether elevated Wnt signaling rescues this osteoblast defect. **Significance:** With a lack of effective anabolic treatments for impaired and difficult to heal fractures, a new therapeutic regimen is needed. This project reveals the positive effects of SostAb on impaired fracture healing in T1DM mice, signifying potential new therapeutic approaches for difficult fracture repairs in a T1DM setting. This also suggests that SostAb treatment, currently in clinical trials for osteoporosis patients, can be repurposed for those who have impaired fracture healing and those with difficult to heal fractures.

**Figure 1.** SclAb treatment improves BV/TV in T1DM. SclAb-treated STZ mice have increased BV/TV in age matched d21 vertebrae by micro-CT.