A sclerostin antibody enhances metaphyseal bone healing in rats

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Introduction:
Most fractures occur in osteoporotic cancellous bone in metaphyseal regions. The response to the trauma of inserting a screw in cancellous bone appears similar to metaphyseal fracture repair. The formation of new bone around a screw determines the strength of its fixation. Hence, the bone regenerative response can be measured as a pull-out force. Sclerostin, a secreted glycoprotein, is the product of the SOST gene. Lack of sclerostin causes increased bone formation and high bone mass in humans. Sclerostin blocks the LRP5/6 receptor, thus antagonizing Wnt signaling and increasing β-catenin degradation. Sclerostin is primarily and probably exclusively expressed in osteocytes, and it is believed to be a potent negative regulator of bone formation. Inhibition of sclerostin by a sclerostin antibody increased bone formation on trabecular, endocortical and periosteal surfaces, and increased trabecular and cortical bone mass \(^1\). A sclerostin antibody enhanced fracture healing in both mouse and rat fracture models \(^2\). We tested the hypothesis that systemic administration of a sclerostin antibody would enhance the regenerative response of traumatized cancellous bone, and thereby the fixation of an implanted screw in non-osteoporotic bone in rats.

Methods:
A murine sclerostin neutralizing antibody (Scl-AbIII) was used. Stainless steel screws were inserted in the proximal tibia of 64 male Sprague Dawley rats, 10 week old with a mean weight of 330 (SD 29) g. The rats were randomly divided into 4 groups of 16 animals. In each group, 12 animals were used for biomechanical testing and 4 were used for morphology. Two groups received subcutaneous injections of 25 mg/kg antibody twice weekly for 2 or 4 weeks. The other 2 groups were administered only saline solution. All animals had a stainless steel screw inserted unilaterally in the right proximal tibia.

Surgical procedure
The rats were anesthetized with isoflurane and subjected to a surgical procedure under sterile conditions. The medial proximal metaphysis was exposed with a longitudinal incision. The periosteum was reflected proximally and an insertion hole was drilled in the cancellous bone, 3 mm distal to the physis a screw was then inserted.

Mechanical testing and ash weight
All analyses were performed while blinded for treatment. The rats were killed using carbon dioxide at the designated time point. 12 specimens from each group were pull-out tested in a materials testing machine. In the untraumatized contra-lateral tibia, a screw was inserted post mortem. All screws were tested for pull-out strength in a materials testing machine. The peak pull-out force was considered the main variable. To determine ash weight, the distal end of the left femur (a 4 mm segment) was incinerated at 900°C for 24 h.

Bone morphology
SEM backscatter images revealed no obvious differences in bone amount or appearance around inserted screws in response to treatment.

Discussion
In our model, a sclerostin antibody improved the fixation of a steel screw in the trabecular bone of young male rats. Even after such a short time span as 4 weeks, the treatment lead to more than 50% increase in pull-out force, as well as significant increases in bone stiffness and pull-out energy. Data from the untraumatized contra-lateral tibia further show that the antibody had a general strengthening effect on the trabecular bone of the proximal tibia. As measured by mechanical resistance, the sclerostin antibodies increased bone formation both during normal remodelling (contralateral tibia) and repair by about half, but the higher background activity during repair made that response 3 times stronger in absolute terms.

Our results are supported by an earlier study in aged osteopenic female rats, using a sclerostin antibody \(^1\). In this study, 5 weeks of sclerostin antibody treatment repealed the negative effects on bone mineral density caused by ovariectomy.

In our model, the inhibition of sclerostin had not as strong anabolic effects as intermittent PTH injections in previous studies using the same model \(^4\). However, these studies used very high doses of PTH, whereas only small doses can be given to humans. The effects such doses of PTH on fracture repair in humans are weak or moderate \(^4\). This dosing situation may be different for antibody treatment.

We believe sclerostin antibodies have a potential to be used to improve the fixation of implants as well as fracture healing.

References
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