Anti-RANKL Treatment Improves Screw Fixation In Cancellous Bone In Rats

Magnus Bernhardsson, Olof Sandberg, Per Aspenberg.
Linköping University, Linköping, Sweden.

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Introduction: Bisphosphonates improve implant fixation in randomized clinical trials of knee prostheses, hip prostheses and dental implants [1-3]. However, a limited amount of bone resorption is required for bisphosphonates to exert an effect [4]. We have previously shown that RANKL inhibition via osteoprotegerin-Fc (OPG-Fc) reduced bone loss in a rat model of prosthetic loosening more potently than high doses of bisphosphonates [5]. We now hypothesized that RANKL inhibition would also improve implant fixation, and compared OPG-Fc with alendronate at very high doses.

Methods: We used bilateral drill holes in the proximal tibiae in 42 male SD rats (age 10 weeks) as a model for cancellous bone healing. Healing of the hole in the left tibia was studied by μCT, and the right tibial hole received a stain-less steel screw for pull-out force measurement as a mechanical estimate of healing. The rats were randomized to subcutaneous injections of either alendronate (20 μg/kg/day), alendronate (200 μg/kg/day), osteoprotegerin with an Fc tag (OPG-Fc; 8 mg/kg, twice weekly), or saline control. After 4 weeks, the fixation of the steel screw was measured by pull-out test (100R, DDL, Eden Prairie, MN). The tibia with the drill hole was evaluated with μCT (1174, Skyscan, Bruker microCT, BE). Statistics: The pull-out force was considered the primary variable. The most important variable from μCT was BV/TV. The analysis was done by t-tests (SPSS v. 22) in sequence, with pairwise comparisons between groups. As soon as the null hypothesis could not be rejected, the sequence of testing was stopped. First we tested the hypothesis that OPG-Fc yields higher results than saline controls. If the hypothesis stood (with a p < 0.05), we tested if OPG-Fc was superior to the high dose alendronate group, and then the extreme dose. BMD was tested as secondary variable.

Results: OPG-Fc increased the pull-out force compared to saline controls by 153 % (p < 0.001). There was no significant difference in pull-out force between OPG-Fc and the alendronate groups. OPG-Fc increased bone volume (BV/TV) in the previous drill hole by 7-fold compared to controls (p < 0.001). This increase was higher than with any alendronate dose (p < 0.001).

Discussion: Anti-RANKL treatment dramatically improved implant fixation. The effect was similar to that of very high doses of alendronate. Because approved doses of bisphosphonates improve clinical implant fixation [1-3] it is tempting to speculate that RANKL inhibition might be even more effective in this context, and perhaps also for aspects of cancellous bone healing.

This study has several weaknesses. Results from rodent models might be relevant for human applications at most regarding biological principles, not regarding practical applications. The drug doses that we used would not be translatable to humans, even if we had had complete dose-response curves. However, with a human intestinal uptake of 1%, the rat doses in mg/kg would correspond to 15 and 150 times human clinical doses. The increased strength of the bone holding the screws in our rats pertains to the response less than 1 mm away from the implant. If the response in humans would be restricted to such a thin layer, it might well be clinically irrelevant. We have not studied the time dependency of the
treatment effect. We have only studied bone by μCT and mechanical testing; detailed histology might provide information regarding soft tissues.

**Significance:** Earlier studies have shown that an anti-RANKL treatment increases the bone formation in non-traumatized cancellous bone. This study shows that an anti-RANKL treatment also increases implant fixation and bone formation in traumatized cancellous bone in rats.

![Image](image1)

**Figure 1.** Pull-out force for metaphyseal screws four weeks after insertion. OPG-Fc increased the pull-out force by 153 % compared to saline controls (p < 0.001). No difference could be seen between OPG-Fc and the alendronate groups.

![Image](image2)

**Figure 2.** μCT bone density analysis of former drill hole in metaphyseal tibia. OPG-Fc significantly increased BV/TV and BMD compared to saline controls and both high dose and extreme dose alendronate (p < 0.001).
Figure 3. μCT of former drill holes in proximal tibia. OPG-Fc resulted in a visibly higher amount of bone in the former drill hole compared to saline controls. No clear difference could be seen between OPG-Fc and the alendronate groups.

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