Effect of Sequential Therapy of Parathyroid Hormone and Sclerostin Antibody for Fracture Healing in Mice

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INTRODUCTION:
The canonical Wnt-beta-catenin (cWnt-β-catenin) pathway plays an important role in bone metabolism, including skeletal development and bone remodeling. It is also known that Wnt pathway genes expression has been found to be upregulated in fracture sites in mice and rats, with the peak of upregulation occurring 7-14 days after fracture, implying the involvement of cWnt-β-catenin pathway in bone healing [1]. Sclerostin, a potent antagonist of Wnt signaling, usually acts as part of a regulatory mechanism of bone metabolism. Inhibiting sclerostin with sclerostin antibodies (Scl-Ab), an anabolic agent for osteoporosis, may promote bone healing, and its efficacy has been demonstrated in multiple animal studies [2]. On the other hand, another anabolic agent, parathyroid hormone (PTH) has also been shown to promote bone healing. Yukata et al. reported that in a mouse fracture model treated with PTH, the expression of the SOST gene (a gene encoding sclerostin) was elevated in the fracture area [3]. From these backgrounds, we considered the possibility that the combination of PTH and Scl-Ab may be more potent in promoting bone healing. The objective of this study is to determine whether the sequential therapy of PTH and Scl-Ab in a mouse fracture model would make a difference in promoting bone healing.

METHODS:
Experimental animals: The experiment was approved by the review board of our institute. C57BL/6J, 8-week-old wild-type, female mice were subjected to unilateral tibia fracture. An open fracture in the proximal tibia diaphysis was performed using a No.11 scalpel blade. An intramedullary pin was inserted into the tibia from the knee to stabilize the fracture. After surgery, mice were divided to six treatment groups: (1) normal saline for two weeks (control group); (2) PTH 1-34 (Teriparatide BS Subcutaneous Injection Kit, MOCHIDA) for two weeks (PTH group); (3) Scl-Ab (Evenity, AMGEN) for two weeks (Scl-Ab group); (4) PTH 1-34 for the first week switching to Scl-Ab for the second week (PTH to Scl-Ab group); (5) Scl-Ab for the first week switching to PTH 1-34 for the second week (Scl-Ab to PTH) group; and (6) simultaneous use of PTH 1-34 and Scl-Ab for two weeks (PTH + Scl-Ab group). Drugs were delivered to the mice by subcutaneous administration. PTH 1-34 was administrated daily with an amount of 40μg/kg per administration and Scl-Ab was administrated twice weekly with an amount of 25mg/kg per administration. Surgery was performed at day 0 and drugs were administrated from day 1 to 14. Mice were sacrificed and specimens were harvested at day 15 for micro-CT and biomechanical testing.
The same surgery and drug administration was done against 1.5-year-old female mice in the following three groups: (1) control group; (2) PTH to Scl-Ab group; (3) Scl-Ab to PTH group. This was done to determine if the results of sequential therapy in older mice showed the same trends as in young mice.
Micro-CT: Specimens were scanned at 2.3-micron isotropic resolution using a CosmoScan GX II (RIGAKU). Volume and bone mineral density (BMD) of external fracture-site mineralized callus was calculated using Analyze 14.0 (AnalyzeDirect).
Biomechanical three-point bending test: Fracture specimens were mounted to EZ-LX 5KN (SHIMADZU) and force was applied from above by a load cell. Three-point bending was applied to the specimen at a rate of 5mm/min until failure to determine loading stiffness and ultimate load to failure.
Statistics:Data are shown as the mean±SE. One-way ANOVA followed by Tukey-Kramer post hoc test was performed using statistical analysis software. *P <0.05 and **P <0.01 vs control group was considered significantly different.

RESULTS:
Micro-CT analysis: In young mice, volume of mineralized callus significantly increased compared to the control group in PTH group, PTH to Scl-Ab group, Scl-Ab to PTH group, and PTH + Scl-Ab group, while Scl-Ab group showed no difference (figure 1A). In contrast, BMD of mineralized callus significantly increased compared to the control group in Scl-Ab group, PTH to Scl-Ab group, Scl-Ab to PTH group, and PTH + Scl-Ab group, while PTH group showed no difference (figure 1B). In sequential therapy, volume of callus was larger in PTH to Scl-Ab group than Scl-Ab to PTH group, although BMD of callus was larger in Scl-Ab to PTH group than PTH to Scl-Ab group. In old mice, similar trend as young mice were seen with PTH to Scl-Ab group showing higher amounts of total volume of mineralized callus and Scl-Ab to PTH group showing higher amounts of BMD of mineralized callus.
Biomechanical three-point bending test: In young mice, loading stiffness significantly increased compared to the control group in Scl-Ab group, PTH to Scl-Ab group, Scl-Ab to PTH group, and PTH + Scl-Ab group (figure 2A). For ultimate load to failure, significant increase against control group was seen in Scl-Ab group, Scl-Ab to PTH group, PTH + Scl-Ab group, while PTH to Scl-Ab group showed no difference (figure 2B). In sequential therapy, loading stiffness showed similar values while ultimate load to failure was larger in Scl-Ab to PTH group than PTH to Scl-Ab group. In old mice, loading stiffness showed no difference between the three groups, though for ultimate load to failure, similar trend was observed as young mice with Scl-Ab to PTH group showing higher value than PTH to Scl-Ab group.

DISCUSSION: In this current study, from the micro-CT analysis results, it was suggested that PTH has a higher proliferative potential of callus and Scl-Ab has a higher mineralization potential of callus. Surprisingly, the results of three-point bending test at this point showed that PTH did not promote bone healing, although callus growth was observed, so it is likely that mechanical strength will increase hereafter. In contrast, Scl-Ab showed early callus mineralization along with promoted bone healing. In sequential therapy, bone healing was more promoted with Scl-Ab first and PTH later than with PTH first and Scl-Ab later. This result supports the results by Kruck B et al. suggesting that Scl-Ab promotes bone formation in the early stages of bone healing, but not in the advanced stages of fracture callus remodeling [4]. The most important limitation for this study is that evaluation was done at a period of two weeks only.

SIGNIFICANCE/CLINICAL RELEVANCE: Our results suggest that Scl-Ab has the ability to promote bone healing early in the fracture phase, and we believe that these results are clinically applicable.

REFERENCES: [1] Zhong N et al. Bone. 2006;39(1):5-16. [2] Mihara A et al. World Journal of Orthopedics. 2021;12(9):651-659. [3] Yukata K et al. Bone. 2014;62:79-89. [4] Kruck B et al. J Bone Miner Res. 2018;33:1686-1697.

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IMAGES AND TABLES:

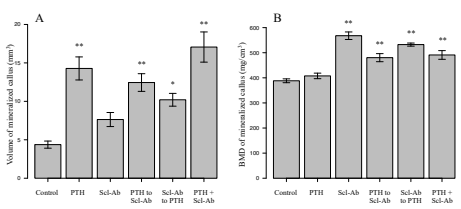

Figure 1. Results of micro-CT analysis of young mice.
A: Volume of mineralized callus. B: BMD of mineralized callus.
*: P<0.05 vs control group. **: P<0.01 vs control group

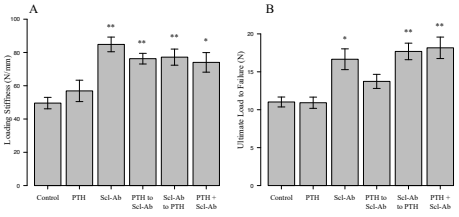

Figure 2. Results of three-point bending test of young mice.
A: Loading stiffness. B: Ultimate load to failure.
*: P<0.05 vs control group. **: P<0.01 vs control group