Vitamin K Maximizes the Efficacy of Teriparatide on Skeletal Repair

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Disclosures:

Introduction: Intermittent administration of PTH1-34, an active recombinant human peptide sequence of parathyroid hormone, has been proven to increase bone mass in patients with osteoporosis. Based on this anabolic property, a number of studies have also shown that PTH1-34 enhances skeletal repair regardless of the skeletal site and mode of bone healing. Since Vitamin K is implicated in bone mineralization by acting as a coenzyme of γ-carboxylase for the Gla protein such as osteocalcin (OC), we hypothesized that vitamin K deficiency or insufficiency caused by malnutrition or therapeutic intake of a vitamin K antagonist warfarin would blunt the efficacy of PTH1-34 therapy on bone repair. We also hypothesized that concurrent use of vitamin K might enhance the efficacy of PTH1-34 therapy for bone repair. To test these hypotheses, we investigated whether the level of vitamin K influences the efficacy of PTH1-34 therapy for bone repair using a rat model of femoral osteotomy.

Methods: Rat model of closed femoral osteotomy: Using female 12 week old Sprague-Dawley rats (n=20), a transverse osteotomized of the mid-shaft femur was performed using the wire saw (MDS36-30 T-saw; MANI, Tochigi, Japan), which was inserted percutaneously. Osteotomy femur was then stabilized with a titanium intramedullary pin (Synthes. Tokyo, Japan).

Experimental design & Treatments: Rats underwent femoral osteotomy and were randomized into four groups: Vehicle, PTH1-34 + solvent, PTH1-34 + warfarin, and PTH1-34 + menatetrenone (Vitamin K2) (each group, n=5). Daily dose of 30 μg/kg/day PTH1-34 (Forteo, Lily Inc., Indianapolis, IN) were administered to the rats via subcutaneous injections for 8 weeks. Solvent or warfarin (0.4 mg/kg/day) or menatetrenone (30 mg/kg/day) were administered by gavage deep into the mouth three times a week. Serum Glu-OC level and the status of bone repair were monitored every two weeks by ELISA and micro-computed tomography (Micro-CT).

Biomechanical testing: Specimens were tested in three-point bending at a rate of 0.2mm/min until failure to determine the stiffness, ultimate load, and energy to failure using a load mechanical universal testing machine (Model 3365, Instron Corp., USA).

Histological evaluation and FTIR analysis: Operative femurs were subjected to undecalcified tissue processing, stained with Villanueva Bone Stain, and evaluated by microscopy. Tissue sections were further analyzed by fourier transform infrared microspectroscopy (FTIR) to evaluate the degree of mineralization and collagen maturity.

Statistical analysis: Statistical analyses were performed with an one-way analysis of variance (ANOVA) and Tukey’s post hoc multiple comparison tests. P values <0.05 were considered statistically significant.

Results: PTH1-34 induced a significant increase in new bone formation and biomechanical properties of osteotomized femur as reported previously. Osteotomy itself (vehicle group) induced 26% increase in Glu-OC from the baseline at 2 weeks after the surgery, while PTH1-34 showed 100-140% increase in Glu-OC from the baseline at 2, 4, and 6 weeks after the surgery (Figure 1). Concurrent use of warfarin blunted the effect of PTH1-34 on bone repair in terms of BMD and biomechanical properties, while concurrent use of menatetrenone increased only biomechanical properties of osteotomized femur (Figure 2). Administration of warfarin demonstrated 40-80% decrease in Glu-OC from the baseline despite the use of PTH1-34, while administration of menatetrenone did not change Glu-OC level compared to PTH1-34 group (Figure 1). Micro-CT analysis failed to detect the significant change by menatetrenone treatment, but histomorphometry and FTIR analysis showed that menatetrenone have additive effect on mineralization of fracture callus. Interestingly, menatetrenone did not further increase Glu-OC level in PTH1-34 treated mice, suggesting that menatetrenone promotes mineralization through the mechanism other than Glu-OC.

Discussion: Although numerous studies have shown that PTH therapy dramatically promotes fracture healing in young healthy rodents, it is still controversial whether PTH improves fracture healing in the elderly or the patients with concomitant diseases. This study showed that concurrent use of warfarin decreased the response to PTH1-34 therapy probably by impairs mineralization due to the lack of Glu-OC, suggesting that the use of warfarin should be avoided or replaced to other anticoagulant when the patient with fracture underwent PTH therapy. In contrast, concurrent use of vitamin K2 was shown to have possibility to provide additive effect on teriparatide therapy for bone repair. Given that vitamin K2, which is a commercially
available drug in the treatment of osteoporosis in Asia, has been reported to suppress bone resorption as well as to stimulate bone formation, it seems reasonable to use vitamin K2 as an adjuvant therapy for teriparatide. In conclusion, vitamin K is required for PTH1-34 to fully exert its effect on skeletal repair and administration of vitamin K2 might further enhance the effect of PTH1-34 on skeletal repair.

**Significance:** In the clinical environment, additional administration of vitamin K2 may further improve bone quality when PTH1-34 is used for bone healing. Furthermore, additional administration of vitamin K2 to fracture patients presenting with high ucOC due to malnutrition or other factors may improve the effects of fracture treatment. However, although administration of PTH to fracture patients who are using warfarin can be expected to increase bone density, the bone healing effect of PTH may not be as strong as previously thought.

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Figure 1: Longitudinal serum Gla-OC level during the bone healing process. *indicates significant difference to the PTH$_{1-34}$ group.

Figure 2: Longitudinal micro-CT scan image during the bone healing process (A), BV (B), BMC (C) and BMD (D) at 8 weeks after operation. *indicates significant difference to the vehicle group.