Enhanced Bone Remodeling Mechanism in Fracture Healing by Low-Magnitude High-Frequency Vibration Treatment

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Introduction
Fracture healing is a complex, highly ordered, physiological process[1]. Bone remodeling is the process performed by the coupled activity of osteoblast and osteoclast in order to maintain bone mass and bone functions[2]. Mechanical stimulation can induce osteogenesis through the process of mechanotransduction[3]. Low-magnitude high-frequency vibration (Vib) is a form of systemic, noninvasive, and cyclic biophysical stimulation. Our previous study showed that Vib (35Hz, 0.3g) improved bone healing, accelerated callus formation, and mineralization in the closed femoral fracture rats[4]. Our hypothesis was callus remodeling is enhanced in Vib during fracture healing. To verify our hypothesis, ibandronate (Bis), a 2nd generation bisphosphonate[5], was used to cause osteoclast apoptosis, and blocking remodeling of callus in the course of fracture healing under Vib stimulation.

Materials and Methods
The Animal Experimentation Ethics Committee of the Chinese University of Hong Kong approved the care and experimental protocols of this study (Ref: 08/026/MIS). Ovariectomy was performed on sixty 6-month old female Sprague-Dawley rats. The rats were housed for 3 months to develop osteoporosis. Closed femoral fracture was created after intramedullary pinning in the right femoral shaft of each rat according our established protocol[4]. The rats were randomly assigned into 4 groups (Control, Vib (20 min/day, 5 days/week), Bis (7 µg/kg/week subcutaneously), and Vib+Bis) at 4 time points (2, 4, 6, and 8 weeks). Both daily Vib and weekly injection of Bis were started 5 days post-operation until euthanasia. Anterior-posterior and lateral x-rays were taken weekly. Callus area and callus width were measured with the lateral x-rays. Bone volume was measured using µCT system (Scanco Medical µCT40). Data was expressed as mean ± 1 SD. One-way analysis of variance (ANOVA) was used to analyze the difference among the four groups, followed by Bonferroni post-hoc test. Two-way ANOVA was used to analyze the main effect and interaction of Bis and Vib treatment. Statistical significant was set at p < 0.05.

Results
Radiologically, Bis showed significantly larger (p=0.05) callus area and width than all groups at all time points. Vib and Bis+Vib showed marginally larger callus measurements than Control in week 3-5. Vib showed the fastest decreasing callus area and width after week 4 and followed by Control; whereas, a plateaued trend for Bis and Bis+Vib. Callus in Vib group were made up of more organized lamellar bone tissue, whereas, a plateaued trend for Bis and Bis+Vib showed the fastest decreasing callus area and width after we started treatment.

Discussion
The callus measurements peaked around week 4 and dropped afterwards suggested that callus remodeling should start around this period. Bis group showed minimal changes in callus measurements because the anti-catabolic effect of Bis increased the net amount of bone at the fracture site and prolonged its retention and delayed the remodeling of the callus. Vib group had larger callus and the fastest drop in callus measurements, which suggested enhanced remodeling and was analogous to our previous study[4]. However, this was not observed in Bis+Vib group. From µCT bone density analysis, the calluses of Bis and Bis+Vib groups were made up of bone that has lower density compared to Vib. A possible explanation for this phenomenon maybe the callus from Bis and Bis+Vib group were made up of less organized woven bone while the callus in Vib group were made up of more organized lamellar bone[6]. In addition, the analysis of interaction of Vib and Bis in 2-way ANOVA suggested that the enhanced callus remodeling effect of Vib was lessened by the presence of Bis. All these results suggested that the enhanced remodeling, caused by Vib, was counteracted by the introduction of Bis in the Bis+Vib group. This might be due to off-balanced osteoblast-osteoclast activity caused by the ibandronate-induced osteoclast apoptosis[2, 7, 8]. This confirmed our hypothesis that Vib accelerated fracture healing by enhanced callus remodeling and the use of ibandronate was able to counteract this enhancement.

The enhanced remodeling from Vib might have great potential in clinical application, allow patient to recover faster and regain function.

References

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