Gene Expression of Osteoporotic Fracture Healing Augmented by Low-Magnitude High-Frequency Vibration Treatment

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Introduction
Accelerating osteoporotic fracture is always a challenge to orthopaedics scientists. Low magnitude high frequency vibration (LMHVF) was reported to have positive influence on bone mineral density, blood circulation, muscle functions and balance control clinically [1,2,3,4] and also in gene expression related to bone metabolism in a rat model[5]. By providing beneficial mechanical strains, LMHVF was found to enhance osteoporotic and normal fracture healing in our previous in vivo studies [6,7]. However, the molecular mechanisms of LMHVF on osteoporotic fracture healing remain unclear. In this study, we hypothesize that LMHVF may promote osteoporotic fracture healing process through up-regulation of osteogenesis, angiogenesis and remodeling-related genes.

Methodology:
Thirty six 9-month-old ovarctomy (OVX)-induced osteoporotic rats were randomized into either control (OVX-C) or vibration group (OVX-V) while 36 sham-operated rats were also assigned into either control (Sham-C) or vibration group (Sham-V). Closed femoral fracture was performed in all of the rats according to our protocol [6]. LMHVF (35 Hz, 0.3g) was then given 20 min/day and 5days/week to the treatment groups, while sham treatment was given to the control groups. Weekly radiographies were taken for monitoring of the fracture healing and the callus width and area of all films were measured [6,7]. At endpoints (week 2, 4 and 8 post-treatment), all of the samples were harvested for RNA extraction and then reverse transcribed to cDNA which were subjected to real-time PCR. Collagen type I (Col-1), collagen type II (Col-2), osteoprotegerin (OPG) and receptor activator for nuclear factor-kappa B ligand (RANKL) were selected as target genes. Gene expression levels of these target genes were relative to that of a housekeeping gene, glyceraldehydes phosphate dehydrogenase (GAPDH). One-way ANOVA with significance level at 0.05 was done for statistical analysis.

Results
Qualitatively, the vibration groups showed faster healing than the corresponding controls from week 4 to 8 (Fig.1). Quantitatively, significantly higher CW and CA of OVX-V than that of OVX-C was shown from week 1 to 3 and from week 1 to 7 respectively (p<0.01 for all). The CA of Sham-V was significantly higher than that of Sham-C on week 1 and 2 (p<0.01 for all). OVX-V also demonstrated higher CW and CA than that of Sham-V on week 2 (p=0.001) and week 1 (p=0.007) respectively but no significant differences were found between OVX-C and Sham-C.

For gene expression, expression level of Col-2 in vibration groups (OVX-V and Sham-V) peaked at week 4 while the control groups (OVX-C and Sham-C) peaked at week 8. At week 2, OVX-V was significantly higher than OVX-C by 1.44 fold (p=0.008) while Sham-V was higher than Sham-C by 1.83 fold (p<0.001). The expression level in OVX groups (OVX-C and OVX-V) was found higher than the Sham groups (Sham-C and Sham-V) from week 2 to 8 post-treatment. OVX-C was significantly higher than Sham-C by 1.82 fold at week 2 (p<0.001) and by 1.52 fold at week 8 (p<0.0002) while OVX-V was also higher than Sham-V by 1.41 fold at week 2 (p<0.001).

For expression level of Col-1, both OVX-V and Sham-V showed higher expression level throughout the healing process than the corresponding control groups (OVX-C and Sham-C). The expression of Col-1 of OVX-V was significantly higher than OVX-C by 3.00 fold at week 2 (p<0.001) and by 1.86 fold at week 8 (p=0.008). Expression level of Sham-V also higher than Sham-C significantly by 2.00 fold at week 8 (p<0.008) and by 1.93 fold at week 8 (p<0.017). When comparing OVX and Sham groups, expression level of OVX-V was significantly higher than Sham-V by 1.83 fold at week 2 (p<0.001). The RANKL/OPG ratio of OVX-V was significantly higher than OVX-C by 1.72 fold at week 8 (p=0.045). Significant differences were also found that OVX-C was higher than Sham-C groups by 1.72 fold at week 4 (p<0.001) and by 1.99 fold at week 8 (p<0.001). No significant differences were found between OVX-V and Sham-V groups.

Discussion
This study was designed to investigate the regulation of gene expression of osteoporotic fracture healing augmented by LMHVF treatment. The results showed that fracture healing in OVX-induced osteoporotic bones (OVX-C) were poor than non-OVX ones (Sham-C) especially in the remodeling stage with low level of RANKL/OPG ratio. Low magnitude high frequency vibration treatment was shown more beneficial to the healing process of non-OVX bones. These findings echoed with our previous study [7]. LMHVF enhanced chondrogenesis in vibration groups by up-regulating the expression level of Col-2 at early stage, as the Col-2 level was peaked at week 4 in vibration groups while it was peaked at week 8 in the corresponding control groups. LMHVF also enhanced mineralization of the callus by up-regulating the expression level of Col-1 in the treatment groups throughout the healing process with significance at week 2 and 8. Expression level of Col-2 and Col-1 of OVX-V group was also significantly higher than Sham-V at week 2. These suggested a more active endochondral ossification process in the vibration group, especially in OVX-C. The previous studies that vibration signals within physiological ranges (0.3g peak-to-peak, 35Hz) [6,7,8,9] stimulated callus mineralization rather than prolonging the chondral phase following a significantly enhanced chondrogenesis process [6,7,10].

The ratio of RANKL/OPG reflects the level of bone remodeling. Significantly lower expression level of the RANKL/OPG ratio was found in OVX-C group than in OVX-V at week 8 and in Sham-C at week 4 and 8. This implied that fracture healing in OVX-induced osteoporotic bones was poorer than non-OVX ones in the remodeling stage, thus delaying the fracture healing process. The ratio of RANKL/OPG of OVX-V group was comparable to Sham-V group and this suggested that LMHVF might also enhance the fracture healing of OVX group at the remodeling stage [7] by regulating the RANKL/OPG ratio. In conclusion, LMHVF enhanced fracture healing and osteoporotic bones indicated more sensitive to the LMHVF than non-OVX bones in the early phase of fracture healing. LMHVF may therefore have great potential in clinical applications for augmentation of osteoporotic fracture healing.

Significance: The study demonstrated that LMHVF enhanced osteoporotic fracture healing by up-regulating the chondrogenesis, osteogenesis and also bone remodeling gene expression. These also help to provide evidences for facilitating clinical trials to apply LMHVF treatment on osteoporotic fracture healing.

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References: