Restoration of Estrogen Receptor Expression for Osteoporotic Fracture Healing by Low-Magnitude High-Frequency Vibration Therapy

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Disclosures:

Introduction: The prevalence of osteoporotic fractures is high and takes longer to heal because healing is impaired at different stages. This multi-factorial problem includes mechanical and biological factors involved in the bone healing process; and is affected by both ageing and estrogen depletion. We have previously reported that Low-Magnitude High-Frequency Vibration (LMHFV) therapy was capable of accelerating fracture healing in ovariectomy-induced osteoporotic fracture healings in rats (1, 2). This biophysical intervention was shown to have enhanced osteoporotic fracture healing in terms of increased callus formation as shown in callus morphometry and gene expression (1, 2). It was also shown that neo-angiogenesis in osteoporotic fracture healing was enhanced that was comparable to non-osteoporotic fractures (3). Since mechanical stimulation can be transmitted by estrogen receptors (ER), and that osteoporotic fracture healing was shown to have altered estrogen receptor expression (4), we hypothesized that the LMHFV therapy may restore ER expressions, contributing to a difference in mechanical sensitivity between normal and osteoporotic fractures.

Methods: Twenty-four 6-month-old SHAM-operated and 72 ovariectomized (OVX) SD rats (PCR: n=5, MicroCT n=3 per group per time-point) were aged for 3 additional months for osteopenia development. Decrease in estrogen levels were confirmed and quantified at endpoint of experiment by enzyme immunonassay (EIA). Each rat then received Einhorn’s closed femoral fracture creation at 9 months of age as previously described. The rats were randomly assigned to each of the 4 groups: SHAM, OVX, OVX-vibration treated at 35 Hz, 0.3g and 20 mins/day, 5 days/week (OVX-VT), OVX-VT-ICI (ER antagonized group by ICI 182,780) and were assessed at week-2, -4, and -8 post-fracture. Total mRNA were extracted from the callus 5-mm above and below the fracture line, and quantified by real-time PCR for the callus formation-related Collagen-1 (Col-1) and Bone Morphogenic Protein-2 (BMP-2) gene expression; ER-alpha, and beta against the reference GAPDH gene. Weekly radiographs were taken for routine monitoring of fracture healing and morphometric measurements of callus width (CW) and callus area (CA). Further callus volumes were assessed by endpoint microCT evaluation of bone volume fracture (BV/TV) at threshold of 165 to 350 to represent the newly formed callus. Differences between groups were compared by one-way ANOVA and post-hoc tests with Bonferroni correction.

Results: ER-α and ER-β gene expressions levels were shown to be similar between the SHAM and OVX-VT groups with no significant difference between two groups. In general, both ERs’ expression levels were higher in the SHAM and OVX-VT group at week 2 and gradually decreased at week 4 and week 8. Whereas the expression of the OVX group showed lower expression at week 2 and later surged at week 8. ER-α expression was suppressed to significantly lower levels in the OVX-VT-ICI group than the other groups, whereas the expression of ER-β was suppressed to lower levels without significant difference when compared to SHAM and OVX-VT group (Figure 1). Similarly, callus formation related Col-1 expression were higher at week-2 (p=0.007) and week-4 (p=0.001) in SHAM and OVX-VT groups; lower in the OVX group and surged at a later time point. BMP-2 expressions were highest in order of the SHAM, OVX-VT, OVX, and OVX-VT-ICI groups at week 4 (p<0.005).

For callus morphometry, SHAM and OVX-VT groups showed peak CW and CA at week 4, while the OVX and OVX-VT-ICI groups peaked at week 6. SHAM and OVX-VT showed higher callus size than OVX group from 2 to 4 weeks (all p<0.05). Both CA were lower in the OVX-VT-ICI group. Significantly higher callus volume was detected by microCT evaluation in the SHAM and OVX-VT groups than the OVX and OVX-VT-ICI groups (p<0.05).

Discussion: Gene expression patterns of ER-α and ER-β in the OVX-VT group took similar apparent trend compared to SHAM group demonstrating the restoration capacity of LMHFV therapy. This finding showed that the presence of ER-α and ER-β expression would return to closer-to-normal levels and may further substantiate the fractured bone’s ability to transmit mechanical strain to stimulate callus formation. This enhancement may have been achieved by improved blood circulation (3) that brings in more reparative stromal cells. These stromal cells expressing ERs (5, 6) might further substantiate the enhancement effects from mechanical stimulation even when estrogen was not abundant, due to ER’s ligand-independent functions in mechanical signal transduction (7). This function of ER in mechanical signal transduction was also shown in this study by the antagonized OVX-VT-ICI group. Gene expression levels of ER-α and ER-β were at very low levels in the OVX-VT-ICI group (8, 9), which is similar to other studies. Enhancement effects by LMHFV therapy on callus size was not observed in the OVX-VT-ICI group, and was found to have similar levels compared the OVX group. Therefore, the presence of ERs was essential in the enhancement effects caused by such vibration stimulation (10).

Significance: Therefore, we conclude that LMHFV therapy could restore the expression of ERs that play an important role in
transducing the vibration signal for an enhancement effect in callus formation during osteoporotic fracture healing.

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**References:**

2. Shi et al, Bone. 2010

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