Low Magnitude High Frequency Vibration Treatment Promotes Osteoporotic Fracture Healing by Enhancing SDF-1 mediated Mesenchymal Stem Cell Recruitment

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Disclosures:

Introduction: Osteoporosis impairs fracture healing. One of the important factors attributed to this impairment is the dramatically decreased number of mesenchymal stem cells (MSCs) in fracture site, which might be partially due to the retarded migration of MSCs through peripheral circulation [1]. This migration effect mainly modulated by stromal cell-derived factor 1 (SDF-1) through its specific receptor, chemokine (C-X-C motif) receptor 4 (CXCR4), is crucial for fracture healing [2,3]. Our previous research confirmed that low magnitude high frequency vibration (LMHFV) could promote osteoporotic fracture healing [4], but the underlying mechanism through which LMHFV enhances the fracture healing is not fully depicted. Therefore, we hypothesize that LMHFV enhances SDF-1 mediated MSC recruitment to accelerate fracture healing in osteoporotic rat model.

Methods: Animal Experimentation Ethics Approval (ref: 11/010/GRF-5) was obtained from The Animal Experimentation Ethics Committee of The Chinese University of Hong Kong before conducting this study. Under general anesthesia, after ovarioectomy, 9-month-old Sprague-Dawley rats (n=120) received a closed transverse femoral fracture, and randomly assigned to 4 groups, consisting of vibration-MSC (VMG), vibration-AMD-MSC(VAMG), MSC(MG) and control group (CG) with 10 animals for each group. For VMG, VAMG and MG, the rats were injected 0.5 mL of 1x10^6 green fluorescent protein labeled MSCs (GFP-MSCs) via left ventricle on day 3 post-fracture. For VAMG, the animals were further injected intraperitoneally with AMD3100 (Sigma, St Louis, MO, USA) 1mg/kg/day and 5 days/week. For VMG and VAMG, daily LMHFV treatment (35Hz, 0.3g) for 20mins/day and 5 days/week was also given till euthanasia at 2, 4 or 8 weeks post-fracture, according to our previous well-established protocols [4]. The rats in the CG were injected with 0.5mL of saline on day 3 post-fracture only. Lateral radiographic analysis was performed weekly for the quantitative measurements of callus width and area. After euthanasia of the animals, the fractured femurs were carefully collected for ex vivo assessment of MSC localization by using fluorescent imaging system (IVIS 200, Xenogen, US). The microarchitecture of the fractured bone was evaluated by micro-computed tomography (μCT) analysis. One-way ANOVA was used to compare the statistical differences among the above measurement variables, followed by Tukey post-hoc test. Statistical significance level was set at p<0.05.

Results: Radiological assessment: Callus width (CW) in VMG were found to be significantly larger than VAMG (p<0.05), MG (p<0.05), and CG (p<0.05) at week 2 and week 3 post-fracture; CW in VMG was significantly smaller than MG (p<0.05) and CG (p<0.05) at week 7 and week 8 post-fracture. CW in VAMG was also significantly smaller than MG (p<0.05) and CG (p<0.05) at week 8 post-fracture. (Fig.1A) Callus area (CA) in VMG was significantly larger than VAMG (p<0.05), MG (p<0.05) and CG (p<0.05) at week 2, week 3 and week 4 post-fracture. CA in VMG was significantly smaller than VAMG (p<0.05) and MG (p<0.05) at week 7 (p<0.05) and week 8 (p<0.05) post-fracture. (Fig.1B) GFP signal intensity analysis: The ex vivo GFP intensity in VMG was significantly increased than VAMG (p<0.005), MG (p<0.005) and CG (p<0.005) at week 2, than MG (p=0.04) and CG (p=0.007) at week 4 post-fracture. μCT analysis: Mean tissue volume (TV) in VMG was significantly larger than VAMG (p=0.006), MG (p<0.005) and CG (p<0.005) at week 2, and significantly larger than VAMG (p=0.023) and CG (p=0.027) at week 4 post-fracture; Mean bone volume (BV) in VMG was significantly larger than MG (p=0.006) and CG (p=0.031) at week 2, and than VAMG (p=0.001) at week 4 post-fracture. Mean bone volume of high-density bone (Bvh) in VMG was significantly larger than MG (p=0.028) and CG (p=0.015) at week 2, and than MG (p=0.044) at week 8 post-fracture. Mean Bvh/TV in VMG was significantly larger than VAMG (p<0.005), MG (p<0.005) and CG (p<0.005) at week 8 post-fracture. Mean BV/TV in VMG was significantly larger than VAMG (p<0.005), MG (p<0.005) and CG (p<0.005) at week 8 post-fracture. Mean bone mineral density (BMD) in VMG was significantly larger than VAMG (p<0.005), MG (p<0.005) and CG (p<0.005) at week 8 post-fracture.

Discussion: SDF-1 was reported to modulate homing and engraftment of circulating MSCs by binding to its receptor CXCR4, thus expanding the pool of osteogenic precursor cells in fracture site [2]. Results of this study confirmed the interactions between LMHFV and SDF-1/CXCR4 pathway in osteoporotic fracture healing. Comparing to MG and CG, vibration increased GFP-labeled MSCs in fracture site that might help promote callus formation at early stage (week 2 and week 4), and later on (week 8) increased BMD in osteoporotic bone. This promoting effect was attenuated when adding SDF-1 antagonist, AMD3100 during LMHFV treatment, as shown by weaker GFP signal intensity in fracture site and smaller callus (week 2 and week 4). The findings of this study showed that mechanical stimulation, in form of LMHFV, might be able to regulate SDF-1/CXCR4 pathway during fracture healing. It also provides strong evidence that SDF-1 mediated MSCs migration might be one of the crucial mechanisms
through which LMHFV enhances osteoporotic fracture healing.

**Significance:** This study explored the mechanism of LMHFV effect on MSCs recruitment in osteoporotic fracture, and also demonstrated the potential of applying LMHFV for promoting osteoporotic fracture healing in the clinical settings.

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**References:**

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