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Low-magnitude high-frequency vibration treatment augments fracture healing in ovariectomy-induced osteoporotic bone

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ABSTRACT

Fracture healing is impaired in osteoporotic bone. Low-magnitude high-frequency vibration (LMHFV) has recently been proven to be osteogenic in osteoporotic intact bone. Our previous study found that LMHFV significantly enhanced fracture healing in adult rats. This study was designed to explore whether LMHFV was able to promote fracture healing in osteoporotic bone by enhancing callus formation, remodeling, and mineralization and to compare with age-matched nonosteoporotic ones. Nine-month-old ovariectomy (OVX)-induced osteoporotic rats were randomized into control (OVX-C) or vibration group (OVX-V); age-matched sham-operated rats were assigned into control (Sham-C) or vibration group (Sham-V). LMHFV (35 Hz, 0.3 g) was given 20 min/day and 5days/week to the treatment groups, while sham treatment was given to the control groups. Weekly radiographs and endpoint micro-CT, histomorphometry, and mechanical properties were evaluated at 2, 4, and 8 weeks post-treatment. Results confirmed that the fracture healing in OVX-C was significantly inferior to that in Sham-C. LMHFV was shown to be effective in promoting the fracture healing in OVX group in all measured parameters, particularly in the early phases of healing, with the outcomes comparable to that of age-matched normal fracture healing. Callus formation, mineralization and remodeling were enhanced by 25–30%, with a 70% increase in energy to failure than OVX-C. However, Sham-V was found to have lesser fracture healing enhancement, with significant increase in callus area only on week 2 and 3 than Sham-C, suggesting non-OVX aged bones were less sensitive to mechanical loading. The findings of this study provide a good basis to suggest that proceeding to clinical trials is the next step to evaluate the efficacy of LMHFV on osteoporotic fracture healing.

Introduction

Primary osteoporosis is a significant co-morbidity that leads to a high incidence of fractures in the elderly population. This is due to the deterioration of bone microarchitecture, decreased bone strength, and increased fragility [1,2]. In the last few decades, osteoporotic fractures have been recognized as one of the most common causes of disability and a major contributor to the rising medical care expenditure [1,3]. Osteoporotic fractures are associated with increased mortalities, especially with osteoporotic vertebral fractures and hip fractures [3–5], which leads to an annual spending of $13.8 billion in the United States [6].

It is widely accepted that fracture healing in osteoporotic bones undergoes the classical stages of the regenerative process which includes hematoma formation, soft callus formation, callus mineralization, and callus remodeling. Meanwhile, a more prolonged and impaired fracture healing process has been shown in animal models following ovariectomy (OVX) [7,8]. Clinically, osteoporotic fractures are associated with an increased rate of implant failure and hampers functional recovery [9,10]. Despite the absence of conclusive clinical studies [11], general consensus is that osteoporosis may disturb the physiological process of fracture healing because of impaired osteoprogenitor cell recruitment [12], the decreased osteogenic capacity with aging [13–15] and reduced osteogenic stimulations from lower physical activities [16,17]. Theoretically, enhancing fracture healing in osteoporotic bones may accelerate the restoration of bony structure, which will decrease the rate of implant failure and allow early rehabilitation of patients.

Low-magnitude high-frequency vibration (LMHFV), a form of biophysical stimulation which provides noninvasive and cyclic mechanical stimulations, has been introduced as a non-pharmacological intervention for osteoporosis [18–20], with proven positive effects on bone mineral density (BMD) [18,20–23], blood circulation in limbs [24], and an increase in muscle fiber area [25,26]. Our previous study used LMHFV to treat fracture healing at the femoral shaft in adult rats, and proved that LMHFV accelerated fracture healing by enhancing callus formation, remodeling, mineralization,

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and mechanical restoration [27]. However, its effects on fracture healing in osteoporotic bones are not known. As osteoporotic bones were reported to be less responsive to mechanical stimulation as compared with normal bones [28–30], it is desirable to study the efficacy of LMHFV on fracture healing in osteoporotic bones.

In this study, we hypothesized that LMHFV was able to accelerate OVX-induced osteoporotic fracture healing through callus formation, remodeling and mineralization. The objective of this study was to investigate the effects of LMHFV on fracture healing in OVX long bone in rat model and also compare with age-matched normal one, with reference to radiography, micro-CT analysis, histomorphometry, and mechanical properties.

Materials and methods

Animal model and experimental design

In total 113 female Sprague–Dawley rats were obtained from the Laboratory Animal Services Center of the Chinese University of Hong Kong, housed and acclimatized at the research animal laboratory. Of the 113 operated rats, seven rats were excluded because of the non-transverse pattern of the fracture line as recommended by others [31]. Two unexpected deaths occurred after fracture. Therefore, 104 rats were available for analysis (72 for radiology/CT/histology and 32 for mechanical test). The Animal Experimentation Ethics Committee of the authors' institution approved the care and experimental protocol of this study (Ref. No. 06/053/MIS).

Nine-month-old rats were then randomly assigned to four groups: (1) sham control (Sham-C), (2) sham vibration (Sham-V) that acted as nonosteoporotic normal group for comparison, (3) ovariectomized control (OVX-C), and (4) ovariectomized vibration (OVX-V), where the rats of the two OVX groups underwent bilateral OVX when the rats were 6-month-old [32,33] and then housed for 3 months to induce osteoporotic conditions [8,34]. A reduction in bone mineral density (BMD) was confirmed by peripheral quantitative computed tomography (pQCT, Densiscan 2000, Scanco Medical, Brüttisellen, Switzerland). BMD drop was detected at the right femoral head, the right femoral shaft and 5th lumbar vertebra by 9.7% ($p=0.005$), 6.6% ($p=0.017$), and 9.4% ($p=0.063$), respectively, as compared with those before OVX. The rats in Sham groups underwent the same surgical procedures without excision of ovaries.

Closed femoral shaft fracture model was established on the rats of all groups using a 3-point bending apparatus following intramedullary insertion of a sterilized Kirschner wire (Sanatmetal Ltd., Eger, Hungary) [27,31]. Briefly, the right knee of the animal was approached through a parapatellar incision. The articular surface of the femoral condyle was exposed following dislocation of the patella. The entry point was anchored in the intercondylar notch, through which, a sterilized Kirschner wire (∅ 1.2 mm) was inserted into the medullary canal retrogradely following reaming with an 18 G needle (∅ 1.27 mm). The K-wire then perforated the proximal femur through the piriformis fossa, and the tip was bent to leave a 3 mm length to prevent distal migration. The distal end of the K-wire was cut at the level of the articular surface to allow free joint movement. After suturing, the rat was then positioned supinely on a customized 3-point-bending apparatus to create fracture in the midshaft of the femur with a metal blade (weighted 500 grams) dropping from a height of 35 cm. A transverse fracture without obvious fracture gap (smaller than 0.5 mm) or displacement (less than 0.5 mm) was confirmed by anteroposterior (A-P) and lateral radiographies. To ensure the consistency, all the animal surgery and fracture procedures were performed by one single experienced orthopedic surgeon. A transverse fracture without obvious fracture gap (smaller than 0.5 mm) or displacement (less than 0.5 mm) was confirmed by anteroposterior and lateral radiographies. Single dose of buprenorphine (0.03 mg /kg, s.c., Temgesic, Schering-Plough, NJ, USA) was given during the first 24 h for analgesia, and the rats were allowed unrestricted cage activities after surgery.

Sham-V and OVX-V rats received LMHFV treatment using a specially designed vibration platform providing vertical vibrations at 35 Hz with a peak-to-peak magnitude of 0.3 g (g=gravitational acceleration), according to our previous protocol [27]. The treatment started at day 5 postoperatively, when the animals were capable of full weight-bearing. The animals were allowed standing separately in bottomless, compartmented cages fixed on the vibration platform for 20 min/day, 5 days/week [27]. On the other hand, Sham-C and OVX-C received sham treatment, with the rats standing on the unpowered vibration platform with the same regime. At 2, 4 and 8 weeks post-treatment, 6 rats from each group were euthanized using overdose of sodium pentobarbital and the femora were immediately harvested for micro-CT and histomorphometric assessments. Additional 8 rats from each group were euthanized for mechanical testing at 8 weeks post-treatment.

Radiographic analysis

Fracture healing status was monitored weekly by anteroposterior and lateral radiography (Faxitron X-ray system model 43855C, Wheeling, IL) with exposure time of 3 s at tube voltage 60 kVp, according to our established protocol [27,35]. Two experienced orthopedic surgeons, who were blinded to the animal grouping and time points of the study, performed independent assessments of the fracture healing status on digitized radiographs for each rat. Radiographic healing was defined as complete mineralized callus bridging of all four cortices on both anteroposterior and lateral radiographs. To quantify and compare the temporal changes of callus morphology, the width (CW) and area (CA) of the radiopaque callus were measured on digitized lateral view images according to our established protocol using Metamorph Image Analysis System (Universal Imaging Corporation, Downingtown, PA, USA) [27]. CW was defined as the maximal outer diameter of the mineralized callus (d2) minus the outer diameter of the femur (d1), while CA was calculated as the sum of the areas of the external mineralized callus.

Micro-computed tomography (micro-CT)

After euthanasia, the fractured femora ($n=6$ for each group at each time point) were harvested for micro-CT scanning (μCT40, Scanco Medical, Brüttisellen, Switzerland) according to our established protocol [27]. The scan range covered 5 mm proximal and 5 mm distal to the fracture line. Contoured region of interest (ROI) was selected in two-dimensional (2-D) CT images with standardized threshold selected according to our established threshold-selecting protocol [27]. Three-dimensional (3-D) reconstruction of mineralized tissue was performed using a low pass Gaussian filter (sigma=1.2, support=2). In the following analysis, the low-density (120–325) and high-density (>325) tissues were reconstructed separately to differentiate the newly formed mineralized callus from the old cortices according to our protocols modified from Gabet's one [27,36,37]. The thresholds were conformed by a series of 2-D and 3-D evaluations with the high-density tissues representing old cortices and newly formed, highly mineralized callus while the low-density tissues representing newly formed callus [27,36,37]. Quantitative analysis was performed covering the mid 300 slices of the 2-D images with the morphometric parameters assessed including total tissue volume (TV, mm^3, calculated from the contoured ROI in 2-D images), volume of high-density bone (BV_h, mm^3), volume of low-density bone (BV_l, mm^3), total bone volume (BV_t, mm^3, equivalent to BV_h+BV_l, or TV − interstitial space) and normalized percentage of the tissue volumes including BV_h/TV, BV_l/TV, and BV_t/TV [27].