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Therapeutic impact of low amplitude high frequency whole body vibrations on the osteogenesis imperfecta mouse bone ☆

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ABSTRACT

Osteogenesis imperfecta (OI) is characterized by extremely brittle bone. Currently, bisphosphonate drugs allow a decrease of fracture by inhibiting bone resorption and increasing bone mass but with possible long term side effects. Whole body mechanical vibrations (WBV) treatment may offer a promising route to stimulate bone formation in OI patients as it has exhibited health benefits on both muscle and bone mass in human and animal models. The present study has investigated the effects of WBV (45 Hz, 0.3 g, 15 minutes/days, 5 days/week) in young OI (*oim*) and wild type female mice from 3 to 8 weeks of age. Vibration therapy resulted in a significant increase in the cortical bone area and cortical thickness in the femur and tibia diaphysis of both vibrated *oim* and wild type mice compared to sham controls. Trabecular bone was not affected by vibration in the wild type mice; vibrated *oim* mice, however, exhibited significantly higher trabecular bone volume fraction in the proximal tibia. Femoral stiffness and yield load in three point bending were greater in the vibrated wild type mice than in sham controls, most likely attributed to the increase in femur cortical cross sectional area observed in the μCT morphology analyses. The vibrated *oim* mice showed a trend toward improved mechanical properties, but bending data had large standard deviations and there was no significant difference between vibrated and non-vibrated *oim* mice. No significant difference of the bone apposition was observed in the tibial metaphyseal trabecular bone for both the *oim* and wild type vibrated mice by histomorphometry analyses of calcein labels. At the mid diaphysis, the cortical bone apposition was not significantly influenced by the WBV treatment in both the endosteum and periosteum of the *oim* vibrated mice while a significant change is observed in the endosteum of the vibrated wild type mice. As only a weak impact in bone apposition between the vibrated and sham groups is observed in the histological sections, it is possible that WBV reduced bone resorption, resulting in a relative increase in cortical thickness.
Whole body vibration appears as a potential effective and innocuous means for increasing bone formation and strength, which is particularly attractive for treating the growing skeleton of children suffering from brittle bone disease or low bone density pathologies without the long term disadvantages of current pharmacological therapies.

Introduction

Osteogenesis imperfecta (OI or brittle bone disease) is a hereditary disease which results in extreme bone fragility. Mutation of the genes coding for collagen type 1 (col-1) is the main cause of OI, resulting in a quantitative or qualitative alteration of col-1 production. This leads to extremely active bone remodelling, disorganized woven bone tissue, reduced trabecular and cortical bone mass and degraded bone mechanical properties [1]. There is currently no direct cure for OI and only symptomatic treatments are available, such as physiotherapy to increase postural strength, surgery to correct bone deformation and bisphosphonate treatment. OI patients treated with bisphosphonates, which reduce the bone resorption, have shown an increase in bone mass and a reduction of fracture and pain [2,3]. Such pharmacologic treatments are now commonly used on children (sometime extremely young) during long periods (2–5 years) with the rationale to maximize the impact on a growing skeleton. However, some concerns have been raised about the equivocal efficiency on the fracture reduction [4,5], the accumulation of those long life drugs and the impact of inhibiting bone remodelling over long periods, which results in the build-up of poor quality, highly mineralized bone [1,6].

It is recognized that the bone tissue is highly responsive to dynamic loading and is able to adapt its architecture and mass to the mechanical loading environment [7–9]. Bone remodelling is sensitive to strain magnitude [10,11], frequency [12,13], number of loading cycles [14], strain rate [15] and rest periods between stimulation [16]. In addition to bone response to high peak strains [17,18], there is also evidence of bone adaptation at low strain but high frequency loading [9,19].

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Because high strain exercises in patient suffering from OI may result in fracture, high frequency low amplitude whole body mechanical vibration (WBV) is an attractive low-impact and drug-free approach to stimulate bone formation. The therapeutic impact of WBV treatment has been observed on muscle strength, motion, posture and bone density in various osteopenic populations: young women [20,21], post-menopausal women [22–25] or children with disabling conditions like cerebral palsy [26] or with OI [27] but no effect has been observed on healthy adults [28]. However more investigations are required to confirm the impact of WBV on bone mass and to identify the most efficient vibration parameters and the most responsive target population [29–33].

Numerous studies have investigated the influence of WBV on bone formation using a large variety of animal models (sheep, rat, mouse) [34–37], age (growing, young or old adults) [38–40], vibration frequency (from 20 to 90 Hz) [41–43], maximum peak acceleration (from 0.1 to 3 g) [43,44], treatment duration (from 10 to 30 min) and treatment length (from 2 weeks to 1 year). A significant osteogenic effect was observed in the trabecular bone of both the femoral condyle and tibial metaphysis of adult sheep (1 year treatment, 30 Hz, 0.3 g) [35,36]. In adult mice, an osteogenic response to WBV is observed in the tibial metaphysis with a non-dose dependent response to acceleration (5 weeks treatment, 45 Hz, 0.1, 0.3 and 1 g) [44]. An influence of the mouse genotype was observed: the osteogenic response to WBV inversely correlated to the low (C57Bl/6J), medium (BALB/c) or high (C3H) bone density of the mouse strain (2 to 3 weeks treatment, 45 Hz, 0.25 g) [37]. An age effect was also observed with no WBV effect on aged BALB/c mice bone and low effects on adult mice (5 weeks treatment, 90 Hz, 0.3 and 1.0 g) [40], while 8 week-old growing mice exhibited a positive response in trabecular and cortical bone [38,39]. Investigations of WBV as a treatment for osteoporosis have shown a positive impact on ovariectomized rats with greatest increase in bone mass at high frequencies [34,41,43] while other investigation reported only an impact on cortical bone [42] or no substantial impact [45]. These variable results suggest a more complex involvement of the hormonal system in the mechanosensitivity of bone to WBV. Interestingly, a positive osteogenic response to "limb vibration" in the absence of weight-bearing has been observed, suggesting an additional mechano-transduction pathway than pure bone strain [9,46].

Previous WBV studies on both patients and animals indicate that vibration is most effective in young growing bone and low density bone. Therefore WBV treatment may offer a promising route to non-invasively stimulate bone formation in OI children. The objectives of the present study were to investigate the effects of WBV on the cortical and trabecular bone formation in growing mice suffering a severe form of osteogenesis imperfecta (*oim* mice).

Materials and methods

Animals breeding and whole body vibration (WBV) protocol

All animal experiments followed the British Home office and institutional guidance (project license 70/6852). 24 Homozygous wild type (B6C3Fe-a/a-+/+) and 24 homozygous *oim* (B6C3Fe-a/a-oim/oim) female mice were bred. Due to a procollagen α2 gene recessive mutation, homozygous *oim* mice produce abnormal homotrimeric collagen type I (Col1-$(\alpha1)_3$) which results in a phenotype mimicking the human type III osteogenesis imperfecta (small body weight, skeletal deformities and brittle bones) [47]. Starting at 3 weeks of age (just after weaning), 12 mice from each genotype group (vibrated groups: Wild vib and *oim* vib) were placed into a custom built WBV transparent plastic cage for 15 min per day, 5 days in a week during 5 weeks. The cage was vibrated vertically at a frequency of 45 Hz and a peak acceleration of ±0.3 g. This vibration regimen was demonstrated to be osteogenic on young growing mice [38,39]. The vibration cage had 8 slots (10∗10 cm each so that 8 mice could vibrate simultaneously) and was mounted on a linear electromagnetic actuator (LAL95-015-70F linear actuator and LAC-1 controller, SMAC Europe Ltd., UK). The linear actuator provided a sinusoidal vertical movement and was force-controlled by a custom made LabVIEW program (NI Corporation Ltd., USA) via a laptop computer and a digital acquisition card (NI USB-6211 multifunction DAQ, NI Corporation Ltd., USA). The actuator was powered by a generator (HY3005D-2, Rapid Electronics Ltd., UK). The acceleration was monitored via an accelerometer (DE-ACCM3D, Dimension Engineering LTD, USA) fixed in the middle of the vibrating cage and the force of the actuator was operator-tuned to obtain a maximum peak acceleration of ± 0.3 g. 12 mice from each genotype group were also placed into the vibrating cage but not subjected to the mechanical vibration (sham groups: Wild sham, *oim* sham). The mice's body weights were recorded during the 5 weeks of vibration treatment.

The mice were injected intraperitoneally with a calcein solution (20 mg/kg) at 10 and 3 days before sacrifice in order to assess bone apposition [48]. Mouse sacrifice was performed by CO_2 asphyxia and the mouse tibiae and femora were dissected and cleaned of soft tissues. The right bones were stored in gauze soaked with phosphate buffered solution (PBS) and frozen at −18 °C. The left bones were fixed in 4% formalin-phosphate buffered solution overnight, rinsed with PBS and stored in 70% ethanol at 4 °C.

3D bone morphology analyses

Right tibiae and femora were scanned using a micro-computer tomography scanner (Metris X-Tek HMX ST 225 CT System) with a 10 μm voxel resolution (80 to 120 kV, 140 μA, 500 μs integration time). Trabecular and cortical bone morphology was analysed in the femur and the tibia using the open source ImageJ software and BoneJ plugin [49]. The cortical bone morphology was analysed (every 10 slices) between 20% and 80% of the femur total length (%TL distal to proximal) and 20% to 90%TL of the tibia after segmenting out the trabecular bone (see Fig. 1). Cortical parameters analysed were as follows: cross section area (CSA, mm^2), minimum and maximum moment of inertia (I_{min}, I_{max}, mm^4) and mean cortex thickness (CtTh, mm). Trabecular bone was analysed (every slice) between 15 and 25%TL in the femur distal metaphysis and between 83 and 93%TL in the tibia proximal metaphysis (see Fig. 1). The trabecular bone was separated from the cortical bone by manually drawing a contour in the proximal tibia while, in the distal femur, an elliptical region of interest (length/width ratio of 1.5) was drawn and replicated every slice. Trabecular bone parameters analysed were as follows: trabecular bone surface (BS, mm^2), trabecular bone volume on total volume (BVTV), mean trabeculae thickness (TbTh, mm) and mean trabeculae space (TbSp, mm).

Three point bending mechanical testing

After CT scanning, right femurs were tested until fracture by three-point bending using a standard materials testing machine (5866 Instron, Instron, Norwood, MA, USA). Femurs were placed on their posterior side on two supports separated by 9 mm and were loaded in the anterior-posterior direction at the mid-diaphysis with a deflection rate of 50 μm/s. Force–deflection curves were analysed with a custom program (Matlab, MathWorks Inc, MA, USA) to measure the bending stiffness (S: slope of the linear elastic deformation), the yield force (F_{yield}, limit between the elastic and plastic deformation) and ultimate force (F_{ult}, maximum force sustained) and the total work to fracture (mJ). The bone elastic modulus E (MPa), ultimate stress σ_{ult} (MPa) and yield stress σ_{yield} (MPa) were calculated using the standard beam theory [50] and the mid femur cross-section dimensions (anteror posterior diameter and medial lateral moment of inertia) measured from the μCT scanner data.