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Research Paper

Bone mineral density, microarchitectural and mechanical alterations of osteoporotic rat bone under long-term whole-body vibration therapy

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ABSTRACT

Low-magnitude, high-frequency whole body vibration (WBV) is receiving increasing interest as a non-pharmacological anti-osteoporosis approach. However, the long-term effect of WBV therapy is seldom studied. In this study, the efficacy of 16-week WBV (0.3 g, 30 Hz) on bone mineral density (BMD), microarchitectural parameters and mechanical properties of ovariectomized rat femur were examined by *in vivo* peripheral quantitative computed tomography (pQCT), *ex vivo* micro-computed tomography (μCT), dynamic mechanical analysis (DMA) and fracture test. To the best of our knowledge, 16 weeks of WBV administration (20 min/day) is currently the longest duration on rodent. The longitudinal BMD change showed that positive effect of WBV on ovariectomized rat femoral neck diminished with prolonged administration duration. In addition, 16-week of WBV treatment was found to cause significantly reduction in the mean BMD, trabecular BMD (Tb.BMD), trabecular bone volume ration (BV/TV), trabecular number (Tb.N) and maximum load in femoral neck of ovariectomized rat. Metaphyseal Tb.BMD and BV/TV were also significantly decreased in WBV treated ovariectomized group than non-treated controls. Whole-femur DMA was demonstrated as a sensitive tool in distinguishing osteoporotic femur from healthy aged-matched controls, in terms of decreased storage modulus (E′) and loss factor (tan δ). However, E′ and tan δ are not enhanced by 16-week WBV treatment. Together, these findings indicate that administration duration played an important role in the effect of WBV. 16-week WBV may exacerbate trabecular bone loss in ovariectomized rat femur, especially in trabecular-rich femoral neck region. An optimal WBV protocol including administration duration should be established prior to long-term clinical practice.

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1. Introduction

Osteoporosis is becoming an important public health issue with the rising elderly population across the world. In postmenopausal women over 50 years of age, bone mineral density (BMD) dropped by 3% per year on average (Dawson-Hughes, 1991). Loss in BMD is strongly associated with an increase in bone fragility and elevated risk of fracture (Brennan et al., 2014). As a non-invasive, non-pharmacological therapy for treating osteoporosis and healing fracture, whole-body vibration (WBV) is increasingly used at homes and in rehabilitation centers (Rubin et al., 2006). Previous research suggests that mechanical stimulation in form of WBV has beneficial effects on the musculoskeleton, including new bone formation (Judex et al., 2007; Rubin et al., 2002), enhanced muscle mass (Nordlund and Thorstensson, 2007; Bogaerts et al., 2007) and stability (Verschueren et al., 2004). Rubin et al. (2001) had established the powerful osteogenic potential of WBV (30 Hz, 0.3 g) on hindlimbs of ovine, which resulted in a 34% higher trabecular bone density in proximal femur than control. It is recognized that during WBV therapy, bone remodeling would be positively activated to adapt to the cyclic mechanical stimuli (Turner, 1998; Ozcivici et al., 2010). And the activation of bone remodeling is highly sensitive to vibratory magnitude (Rubin and Lanyon, 1985), frequency (Warden and Turner, 2004) and time course administered (Srinivasan et al., 2007).

To date, the initial attempts to increase BMD in osteoporotic bone using WBV therapy were not uniformly successful. Previous studies quantifying BMD in standard ovariectomized rodent model have found increases (Sehmisch et al., 2009; Tezval et al., 2011; Judex et al., 2007), decreases (Pasqualini et al., 2013), whilst others present no alterations in mineral content (van der Jagt et al., 2012; Brouwers et al., 2010) as a consequence of WBV therapy. In clinical trials, widely variable WBV parameters (magnitude, frequency and duration) were used in different protocols which complicate study interpretation. It is generally believed that the effect of WBV on osteoporotic bone highly depends on vibratory parameters used and closely related to the physiological site (Pasqualini et al., 2013). While most of the researchers have focused on optimizing frequency and magnitude used in WBV therapy to render best result, few explored the relationship between the effect and the duration administered. Furthermore, long-term effect of WBV on bone is seldom studied. No data to date have reported effect of WBV applied on ovariectomized rodent model of longer duration than 3 months. In fact, chronic vibratory stimuli have for long been associated with musculoskeleton disease, *i.e.*, low back pain of drivers (Tiemessen et al., 2008) and construction workers (Miyashita et al., 1992). Since WBV is increasingly being prescribed to postmenopausal women with unclear administration duration, it is imperative to identify safety concerns relate to its long-term usage.

Thus, the primary aim of this investigation was to evaluate the long-term effect of WBV on osteoporotic bone. We hypothesized that 16 weeks of WBV intervention would reduce bone loss in ovariectomized rat femur and eventually improve static and dynamic mechanical properties of bone. A 4-week interval *in vivo* Peripheral quantitative computed tomography (pQCT) scanning was conducted to monitor the longitudinal areal BMD changes induced by WBV administration. At the endpoint, *ex vivo* micro-computed tomography (μCT) was used to characterize volumetric BMD, trabecular bone microarchitectural change in femoral neck and metaphysis, as well as geometrical change in femur diaphysis. Femoral neck fracture test was done to assess the effect of WBV on bone load-bearing ability. Furthermore, bone viscoelasticity of rat skeleton which received long-term WBV is expected to be changed. Viscoelasticity of bone mainly arises from natural viscoelastic response of collagen, as well as void collapse and densification of trabecular bone (Cowin., 1999; Garner et al., 2000). Previous studies have showed that bone collagen secretion (Ignatius et al., 2005; Tirkkonen et al., 2011) and trabecular bone quality were positively altered by vibration stimuli (Judex et al., 2007; Rubin et al., 2002). Thus in the current study rat femur with vibration treatment was presumed to damp oscillatory stress more efficiently. Dynamic mechanical analysis (DMA) was used here to assess the effect of WBV on bone damping ability (viscoelasticity). DMA has been proved to be a sensitive tool in distinguishing different physiological directions, nutritions and diseased conditions of the bone sample derived (Abdel-Wahab et al., 2011; Chang et al., 2011; Les et al., 2005). In previous study, we have successfully established a method using DMA to assess osteoporotic drug efficacy (Yang et al., 2013b), *i.e.*, parathyroid hormone, ibandronate. For the present study, we plan to take one step further by using the tissue-level non-destructive whole-femur DMA approach.

2. Materials and methods

2.1. Experimental design and animal model

All procedures in the experiment were approved by the Animal Care and Use Committee of Sichuan University. Twenty-four mature female Sprague-Dawley (SD) rats at the age of 3 months were obtained from Laboratory Animal Center of Sichuan University (Chengdu, China). Rats were acclimatized for two weeks under standard condition of temperature (25 °C) and light-controlled environment (12 h light/dark cycles). Four weeks prior to week 0, all 24 rats were subjected to ovariectomy or sham surgery and evenly distributed into the following 3 groups (n=8/group): (1) sham surgery operated rats without treatment (SHM); (2) ovariectomized rats without treatment (OVX); (3) WBV treated ovariectomized rats (OVX+W). Starting from week 0, a 0.3 g, 30 Hz, 20 min/day WBV treatment were administered to OVX+W group via a commercial vibrating platform (Juvent Medical Inc., Somerset, NJ) for the next continuous 16 weeks. The SHM and OVX rats were taken out of the cages and placed on the vibration platform without vibration, 20 min/day. At the end of week 16, animals were euthanized by carbon-dioxide asphyxiation. Whole femur bone were harvested, wrapped in 0.9% saline soaked gauze and stored at −20 °C until they were used for the experiments.