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Mechanical vibration inhibits osteoclast formation by reducing DC-STAMP receptor expression in osteoclast precursor cells

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ABSTRACT

It is well known that physical inactivity leads to loss of muscle mass, but it also causes bone loss. Mechanistically, osteoclastogenesis and bone resorption have recently been shown to be regulated by vibration. However, the underlying mechanism behind the inhibition of osteoclast formation is yet unknown. Therefore, we investigated whether mechanical vibration of osteoclast precursor cells affects osteoclast formation by the involvement of fusion-related molecules such as dendritic cell-specific transmembrane protein (DC-STAMP) and P2X7 receptor (P2X7R).

RAW264.7 (a murine osteoclastic-like cell line) cells were treated with 20 ng/ml receptor activator of NF-κB ligand (RANKL). For 3 consecutive days, the cells were subjected to 1 h of mechanical vibration with 20 μm displacement at a frequency of 4 Hz and compared to the control cells that were treated under the same condition but without the vibration. After 5 days of culture, osteoclast formation was determined. Gene expression of DC-STAMP and P2X7R by RAW264.7 cells was determined after 1 h of mechanical vibration, while protein production of the DC-STAMP was determined after 6 h of postincubation after vibration.

As a result, mechanical vibration of RAW264.7 cells inhibited the formation of osteoclasts. Vibration down-regulated DC-STAMP gene expression by 1.6-fold in the presence of RANKL and by 1.4-fold in the absence of RANKL. Additionally, DC-STAMP protein production was also down-regulated by 1.4-fold in the presence of RANKL and by 1.2-fold in the absence of RANKL in RAW264.7 cells in response to mechanical vibration. However, vibration did not affect P2X7R gene expression. Mouse anti-DC-STAMP antibody inhibited osteoclast formation in the absence of vibration.

Our results suggest that mechanical vibration of osteoclast precursor cells reduces DC-STAMP expression in osteoclast precursor cells leading to the inhibition of osteoclast formation.

Introduction

Bones are subjected to a variety of mechanical loads during daily activities. Bone mass and architecture are continuously adapted to the daily mechanical loads. Osteocytes play an important role in the adaptation of bone to mechanical loading by sensing the mechanical loads [1,2]. Osteocytes are thought to regulate bone mass by orchestrating the balance between bone formation and resorption in response to mechanical cues, such as vibrations. Mechanistically, osteoclastogenesis and bone resorption have recently been shown to be regulated via osteocytes in response to vibration [3]; however, this result leaves an interesting question, whether osteoclastogenesis is directly regulated by vibration. Lately, Wu et al. have shown that low-magnitude high-frequency vibration (0.3 g, 45 Hz) of osteoclast precursor cells inhibits the formation of osteoclasts [4]. However, the underlying mechanism behind the vibration induced inhibition of osteoclast formation in osteoclast precursor cells is yet unknown.

Recent studies suggest that vibration can positively influence skeletal homeostasis. Animal studies have demonstrated that vibration (0.3 g, 30 Hz; 0.15 g, 90 Hz; 0.3 g, 45 Hz) stimulated an anabolic response in both weight-bearing [5,6] and non-weight-bearing [7] bones. Moreover, vibration (3.0 g, 45 Hz; 0.3 g, 45 Hz) prevented mice from ovariectomy-induced osteoporosis [8] and decreased osteoclast activity in the adolescent mouse skeleton [9], providing evidence of vibration's anti-resorptive potential. Whole-body vibration (0.2 g, 30 Hz; 2.2–5.0 g, 35–40 HZ) of human subjects was found to be anabolic to the bone in vivo, as postmenopausal women treated with vibration stimulation gained higher bone mineral density (BMD) in hip and spine compared to the placebo group after 6–12 months [10,11]. In dentistry, physiological vibrational (chewing) load is able to maintain alveolar bone in mice [12] and rehabilitate resorbed alveolar bone in rats [13]. Although the anabolic and anti-resorptive potential of vibration is becoming apparent, it is imperative to study the underlying mechanism

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behind the direct effect of vibration on osteoclast formation in osteoclast precursor cells.

Osteoclasts are multinucleated cells that arise from hematopoietic cells of the monocyte/macrophage lineage [14,15]. The process of osteoclast formation is composed of several steps, including progenitor survival, differentiation of mononuclear pre-osteoclasts, fusion of multi-nuclear mature osteoclasts, and activation of the bone resorbing osteoclasts. The regulation of osteoclast formation has been extensively studied in which the receptor activator of NF-κB ligand (RANKL)-mediated signaling pathway and downstream transcription factors play essential roles [16]. Several proteins that affect cell fusion have been identified. Among them, dendritic cell-specific transmembrane protein (DC-STAMP) is directly associated with osteoclast fusion in vivo. DC-STAMP has a seven-transmembrane domain structure similar to the members of the G protein-coupled receptor (GPCR) superfamily. Among the GPCR superfamily, the CCR5 chemokine receptor is known to function as direct cell adhesion molecules through interactions with their transmembrane-type ligands. Given the structural similarity between DC-STAMP and chemokine receptors, DC-STAMP may also function as a direct cell adhesion molecule by interacting with its ligand. The osteoclast fusion process may be initiated upon this adhesive interaction. The ligand for DC-STAMP may be membrane bound or soluble; a soluble ligand might be released by either of the fusion partners. DC-STAMP ligation may trigger fusion of the two cells directly or may trigger the expression of as yet unknown membrane-bound molecules ('X') that mediate fusion [17,18]. DC-STAMP expression was rapidly up-regulated when mouse cells were cultured in the presence of osteoclast-promoting cytokines such as RANKL [17], and inhibition of murine DC-STAMP with a polyclonal antibody suppressed osteoclast formation [17]. Consistent with these observations, the phenotype of DC-STAMP knockout mice shows few multinucleated osteoclasts and increased bone mass [16]. In contrast, overexpression of DC-STAMP in mice resulted in a phenotype with accelerated cell-to-cell fusion during osteoclast precursors' differentiation and enhanced bone resorption [19].

Moreover, the P2X7 receptor (P2X7R) has been implicated in the process of multinucleation and cell fusion. The P2X7R is a 595 amino acid plasma membrane receptor with approximately 40% sequence identity to other members of the P2X purinergic family [20]. It has been shown that blockade of P2X7R on osteoclast precursors using a blocking antibody inhibited multinucleated osteoclast formation in vitro [21]. Conversely, P2X7R deficient mice maintain the ability to form multinucleated osteoclasts [22].

In the present study, we studied the effect of vibration on osteoclast formation by subjecting osteoclast precursor RAW264.7 cells to vibration with a magnitude of 20 μm displacement at a frequency of 4 Hz. The aim of this study was to investigate whether mechanical vibration of RAW264.7 cells affects osteoclast formation by the involvement of fusion-related molecules such as DC-STAMP and P2X7R.

Material and methods

Cell culture

RAW264.7 (ATCC, Manassas, VA) cells between 10 and 14 passages were used for the osteoclast formation assay. RAW264.7 cells were cultured up to near-confluency in 75 cm^2 culture flasks using α-MEM supplemented with 10% fetal bovine serum (ATCC, Manassas, VA), 100 IU/ml penicillin and 100 μg/ml streptomycin (Cellgro, Manassas, VA) at 37 °C and 5% CO_2 in air.

Mechanical vibration setup

The experimental setup outlined in Fig. 1 contained both mechanical and electrical components that interface to form a complete mechatronic test system. The core of the mechanical component was a ThorLabs Max Series Modular Flexure Stage (MAX303) with a DRV120 actuator. The output of the stage was connected to an adapter plate which fits the regular cell culture plate. The core of the electrical system was a laptop running National Instruments (NI) Labview. The interface between the computer and all electrical components was done through an NI USB-6211 multifunction bus powered data-acquisition box (DAQ). The DAQ provided the input signal to a closed-loop piezo controller (Thorlabs BPC201) specifically designed for the DRV120 actuator. The controller provided the necessary control signal (including amplification) to the piezo. All high level control to the system was made through a custom graphical user interface (GUI) in Labview. After starting the GUI, the user chose the signal type (sinusoidal, square, trapezoidal), amplitude, and frequency as well as the total testing time. In this study, we specifically chose sinusoidal wave, a magnitude of 20 μm displacement (peak to peak), a frequency of 4 Hz and a total testing time of 1 h. The entire mechanical component of this

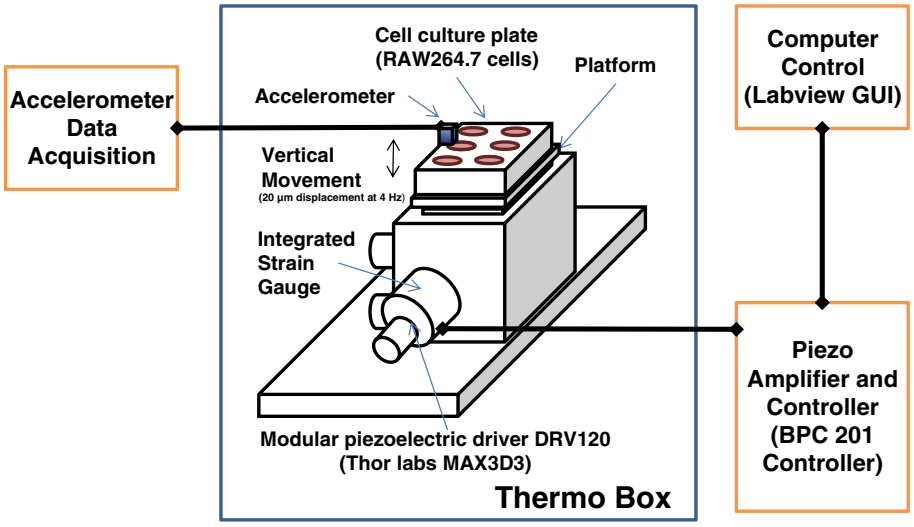

Fig. 1. Experimental setup. A rigid platform was custom-made to fit a standard multi-well tissue culture plate. A vertical vibration (20 μm displacement at 4 Hz) was generated by a modular piezoelectric device and controlled by a piezo amplifier and controller connected to a computer equipped with a VibeLab user interface. An accelerometer was attached to the culture plate to confirm the vibration received by the cells. The mechanical component was housed in a thermo box at 37 °C.