

Mechanical Regulation of Bone Homeostasis: Effects of Cyclic Pressure on Bone Cell Function *in Vitro*

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Abstract – The present *in vitro* study exposed rat osteoblasts and osteoclast-precursors in bone marrow cell populations to controlled regimes of cyclic pressure and examined various cell functions that are pertinent to bone homeostasis. The results provided evidence that osteoblasts are sensitive to the frequency of the applied cyclic pressure stimulus. Specifically, compared to controls (static conditions) and cells exposed to cyclic pressure at 0.25 Hz frequency, osteoblast proliferation was significantly ($p < 0.05$) lower, but alkaline phosphatase mRNA expression and enzyme activity were enhanced, only when these cells were exposed to cyclic pressure at 1.0 Hz frequency for 1 hour daily for 5 consecutive days. Furthermore, the results of the present study demonstrated that the timing of application of the cyclic pressure was critical for osteoclastic cell formation from precursors in bone marrow. Exposure of bone marrow cells to cyclic pressure immediately upon harvesting led to decreased formation of osteoclastic cells from their precursors; in contrast, the number of osteoclastic cells was not affected when the cyclic pressure was applied after 7 days of culture under static conditions.

INTRODUCTION

The motivation for the present study was information from clinical studies reported in the literature that prolonged bed rest and absence of mechanical loading on the skeleton caused up to 10% bone loss in healthy human subjects [1]. Another study reported that under conditions of weightlessness such as encountered during long-term spaceflights astronauts lost 250 mg of bone calcium per day [2]. In contrast, increased loading on the skeleton through exercising led to higher bone mineral density in both aging and young human subjects [3, 4]. These and similar studies provided evidence that there is a relationship between mechanical loading on the skeleton and bone homeostasis. To date, however, the mechanisms that link mechanical stimuli and bone homeostasis, especially at the cellular- and molecular level, are yet to be elucidated.

In the present *in vitro* study, a custom-made laboratory setup was used to expose bone cells, namely, osteoblasts and bone marrow cells, to cyclic pressure; cell proliferation,

mRNA expression and activity of alkaline phosphatase by osteoblasts as well as formation of osteoclasts from the bone marrow precursor cells under controlled regimes of cyclic pressure were examined.

METHODS

Osteoblasts were isolated from the calvariae of neonatal Sprague-Dawley rats by enzymatic digestion and cultured following established and published methods [5]. The osteoblastic phenotype of the cells was determined by expression of genes for collagenous and non-collagenous proteins present in the bone matrix and formation of calcium phosphate mineral deposits in the extracellular matrix. Bone marrow cells (used as a source of osteoclast precursors) were harvested from the femurs of 14 day old Sprague-Dawley rats and cultured using a method adapted from the literature [6]. Osteoclastic cells were identified by their large multinucleated morphology and positive staining for tartrate resistant acid phosphatase (TRAP).

A custom-made, computer-operated, cyclic pressure system was designed, assembled and used in the present study to expose osteoblasts and bone marrow cells to cyclic pressure [7]. Osteoblasts (in DMEM containing 10% FBS) and bone marrow cells (in DMEM containing 10% FBS and 10 nM 1,25-(OH)₂ vitamin D₃) were cultured separately on etched glass coverslips and were exposed to cyclic pressure (10 – 40 kPa at either 0.25 Hz or 1.0 Hz frequency) for 1 hour daily for up to 7 consecutive days. Controls were similar cell preparations maintained under standard (no pressure) cell culture conditions for the duration of the experiments.

At the end of the prescribed time periods, osteoblasts in control and in cultures exposed to cyclic pressure were lysed and the released nuclei were counted using a hemocytometer. Messenger RNA expression for alkaline phosphatase was determined using Reverse Transcriptase – Polymerase Chain Reaction (RT-PCR) techniques and enzyme activity of alkaline phosphatase in cell lysates was

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determined according to methods adapted from the literature [8]. Formation of osteoclastic cells from bone marrow cells was quantified by counting multi-nucleated, TRAP-positive cells.

RESULTS AND DISCUSSION

The results of the present study demonstrated that cyclic pressure affected important functions of osteoblasts. Specifically, there was similar osteoblast proliferation in controls and in specimens exposed to cyclic pressure at 0.25 Hz frequency for 1 hour daily for up to 5 consecutive days. In contrast, and compared to controls, osteoblast proliferation was significantly ($p < 0.05$) lower when these cells were exposed to cyclic pressure at 1.0 Hz frequency for 1 hour daily for 5 consecutive days [7]. Furthermore, the effects of cyclic pressure on both activity and mRNA expression for alkaline phosphatase (an enzyme important for bone formation [9]) were examined. The results of RT-PCR revealed similar expression of alkaline phosphatase gene for osteoblasts maintained under control conditions and osteoblasts exposed to cyclic pressure at 0.25 Hz frequency for 1 hour daily for 5 consecutive days. In the cells exposed to cyclic pressure at 1.0 Hz frequency (but otherwise similar cell culture conditions), however, there was enhanced expression of alkaline phosphatase mRNA at 5 days. Enhanced gene expression does not necessarily mean enhanced synthesis of alkaline phosphatase because transcription and translation are two separate events. For this reason alkaline phosphatase activity in osteoblasts exposed to cyclic pressure was examined. Alkaline phosphatase activity in lysates of osteoblasts exposed to cyclic pressure followed a trend similar to the results of alkaline phosphatase mRNA expression; compared to controls and to cells exposed to pressure at 0.25 Hz, there was increased alkaline phosphatase activity in osteoblasts exposed to cyclic pressure at 1.0 Hz for 1 hour daily for 5 consecutive days. These results indicate that osteoblasts are sensitive to the frequency of the applied cyclic pressure stimulus and that cyclic pressure triggers cellular/molecular events pertinent to new bone formation.

The present study also examined the effects of cyclic pressure on osteoclasts and provided evidence that the timing of application of the cyclic pressure was critical for osteoclastic cell formation from bone marrow precursor cells. Specifically, exposure of bone marrow cells to cyclic pressure applied immediately upon harvesting bone marrow cells inhibited formation of osteoclastic cells. When, however, the cyclic pressure was applied to bone marrow cells after 7 days under standard cell culture conditions the numbers of osteoclastic cells were similar to those of controls.

In summary, cyclic pressure affects bone cell functions that are important in bone homeostasis. The results of the present study, therefore, not only make contributions to

bone cell physiology, but also provide cellular/molecular level insights into the relationship between mechanical loading and bone homeostasis.

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