REGULATION OF THE RANKL/OPG RATIO BY FLUID SHEAR STRESS in MC3T3-E1 CELLS


*Center for Orthopaedic Research & Department of Orthopaedic Surgery, Columbia University, New York, NY, *Department of Electronic Engineering, Hallym University

630 West 168th Street, BB14-1412, New York, NY 10032
fl127@columbia.edu

INTRODUCTION

Bone is known to adapt to mechanical loading in order to maintain its structural and functional integrity (1-3). Particularly, fluid shear stress (FSS) in response to mechanical loading is known to trigger expression of key effectors involved in osteoblastic and/or osteoclastic cellular activity (1-3). In order to examine whether FSS may regulate osteoclastic activity by modulating the receptor activator of NF-κB ligand (RANKL) to osteoprotegerin (OPG) ratio via osteoblasts, we studied the RANKL/OPG ratio in MC3T3-E1 pre-osteoblasts exposed to various regimens of FSS (Figure 1). The RANKL/OPG ratio, which is influenced by FSS, enables us to evaluate the effects of FSS on osteoclastogenesis, since a high RANKL/OPG ratio is known to enhance osteoclastogenesis. Furthermore, no detailed study has been performed to investigate the RANKL/OPG ratio using MC3T3-E1 cells subjected to various regimens of FSS. The RANKL/OPG ratio was significantly decreased as duration of FSS in osteoblasts. Here, we report that FSS differentially regulated the RANKL/OPG ratio. Our data may partly explain why exercise is beneficial for bone health. Next, we will focus on examining how osteoblastic activity is influenced by FSS.

MATERIALS AND METHODS

Cell Culture: MC3T3-E1 pre-osteoblasts purchased from the American Type Culture Collection (ATCC, Manassas, VA) were maintained in α-MEM with 10% FBS for 24 hr at 37 °C in a humidified atmosphere of 5% CO₂. Cells were seeded onto the culture slips prior to FSS and maintained in the same medium before use.

Fluid Shear Stress Regimen: FSS was applied to each cell on the culture slips using a STREAMER™ system (Flexcell International Inc, Hillsborough, NC) under the control of STREAMER Soft™ software. 0, 1, 5, and 10 dynes/cm² at 1 Hz (bipolar pulse) were applied to each cell for 15 min. 0, 0.5, 1, and 2 Hz at 5 dynes/cm² were applied to each cell for 15 min. Various durations including 0, 15, 30, and 60 min were also applied to each cell using 5 dynes/cm² at 1 Hz. Cells were harvested 24 hours after FSS, and RANKL and OPG protein expressions were examined by immunoblotting.

Immunoblotting: MC3T3-E1 cells subjected to FSS were immunoblotted and incubated with the following antibodies: RANKL (Calbiochem, San Diego, CA), OPG (R&D Systems, Minneapolis, MN) and GAPDH (Chemicon, Temecula, CA). Each band in the immunoblots was quantified using NIH image software. The normalized band intensities with GAPDH were plotted.

RESULTS

1. The RANKL/OPG ratio decreased as the magnitude of FSS increased.

In order to examine whether different FSS magnitudes may differentially affect the RANKL/OPG ratio, 0, 1, 5, and 10 dynes/cm² at a frequency of 1 Hz were applied to MC3T3-E1 cells on culture slips for 15 min. Both RANKL and OPG expression levels increased in a strain magnitude-dependent manner from 0 to 10 dynes/cm², and the resulting RANKL/OPG ratio decreased as the magnitude of FSS increased (Figure 2).

2. The RANKL/OPG ratio decreased as the duration of FSS increased.

In order to examine whether the differences in duration may differentially affect the RANKL/OPG ratio, FSS of 5 dynes/cm² at 1 Hz was applied to MC3T3-E1 cells on culture slips for 0, 15, 30, and 60 min. The level of RANKL protein decreased as the duration of FSS increased from 0 to 60 min, while OPG protein expression was not significantly altered by the differences in duration. The resulting RANKL/OPG ratio was found to decrease as the duration of FSS increased from 0 to 60 min (Figure 3).

DISCUSSION

In this study, we investigated the role of FSS in regulating the RANKL/OPG ratio using MC3T3-E1 cells subjected to various regimens of FSS. The RANKL/OPG ratio was significantly influenced by both the differences in FSS magnitude and duration. The RANKL/OPG ratio at a shorter duration/high magnitude regimen at 10 dynes/cm² at 1 Hz for 15 min turned out to be 0.5, while the RANKL/OPG ratio at a longer duration/low magnitude regimen at 5 dynes/cm² at 1Hz for 60 min turned out to be 0.8. This suggests that a high impact, low duration exercise regimen is more effective than a low impact, high duration exercise regimen in preventing bone loss. Further studies are needed to confirm this notion.

REFERENCES