INTRODUCTION

Mechanical loading stimulates bone formation. Loading of bone results in localized pressure gradients that drive interstitial fluid flow, which is oscillatory by nature, within the bone tissue [1]. Oscillatory fluid flow (OFF) induced shear stress has been shown to influence osteoblastic differentiation through increases in intracellular calcium mobilization and osteopontin gene expression in MC3T3-E1 pre-osteoblasts [2]. Stromal/osteoblastic lineage cells have also been shown to affect osteoclast differentiation by expressing the receptor activator of NFκB ligand (RANKL), an essential requirement for osteoclastogenesis, and osteoprotegerin (OPG), a decoy receptor that neutralizes the activity of RANKL [3,4,5]. However, the effect of OFF on stromal/osteoblast cells that regulate osteoclastic differentiation at the cellular level is not fully understood.

Therefore, in this study, we investigated the effects of various durations of OFF on expression of RANKL and OPG, two important regulators of osteoclastogenesis.

MATERIALS AND METHODS

Cell Culture: ST-2 murine bone marrow stromal cells were obtained from Riken (Japan) and cultured at 37°C with 5% CO₂. Culture medium was αMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. 48 hours prior to the onset of flow experiments, cells were seeded on sterile glass slides (75 mm x 38 mm x 1 mm) at a density of 700,000 cells/slide and cultured in medium supplemented with 10 nM 1α,25-dihydroxyvitamin D₃ (Fluka, Switzerland) for expression of RANKL.

Oscillatory Fluid Flow: 48 hours after plating of cells, the glass slides were placed in custom-designed sterile parallel plate flow chambers [2]. OFF was delivered to the cells via syringes connected to an electromagnetic actuator (ELectroForce Actuator, EnduraTEC, Minnetonka, MN). Cells were exposed to a maximum of ±10 dynes/cm² at 1 Hz using a sinusoidal waveform for (1) 30 minutes, (2) 1 hour, or (3) 2 hours. Control groups for each time period were also placed in parallel plate flow chambers with no flow applied. Each of the 6 groups consisted of at least 3 samples.

Real Time RT-PCR: Immediately after end of flow, total RNA was isolated using Triz-Reagent (Sigma, St. Louis, MO). RNA was reverse transcribed to cDNA and real time RT-PCR was performed for quantification of RANKL and OPG (Applied Biosystems, Foster City, CA) expressions in triplicates. Each gene was normalized by 18S.

Statistics: Results were analyzed using Student’s t-test with significant difference assumed at p<0.05. Error bars represent standard deviation.

RESULTS

OFF resulted in a significant increase in OPG (Fig. 1) and a significant decrease in RANKL (Fig. 2) compared to no flow controls. The change compared to controls was greater with longer durations of OFF. The RANKL/OPG ratio significantly decreased when cells were exposed to OFF for 30 minutes, 1 hour, as well as 2 hours (Fig. 3).

DISCUSSION

Consistent with a previous study where a uniform biaxial 1.8% cyclic strain on primary stromal cells for 6 hours decreased the RANKL expression by 40% [6], our results indicate that OFF can induce a similar decrease within 2 hours of OFF. Interestingly, OPG was not upregulated with strain in the same study whereas we demonstrate significantly increased OPG level with only 30 minutes of OFF. This suggests that OFF induced shear stress and a direct mechanical strain on the cell may not follow the same mechanotransduction pathway.

There was a synergistic effect of upregulation of OPG and downregulation of RANKL which resulted in the RANKL/OPG ratio decreasing by 60% after 30 minutes of OFF and by 90% after 2 hours of OFF compared to corresponding no flow controls. As a change in RANKL/OPG ratio has been shown to have a direct impact in osteoclast formation in vitro [5], our results suggest that OFF induced cyclic shear stress may be an important mechanical signal that could lead to positive net bone balance not only through increases in osteoblastic activity but also through significant decreases in osteoclastogenesis.

Acknowledgments

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References