EFFECTS OF OSCILLATORY AND PULSE-STATIC SHEAR ON ACTIVATION OF NF-KB, C-FOS, AND SRF IN MC3T3 OSTEOBLAST-LIKE CELLS

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INTRODUCTION

Bone growth, repair, and homeostasis are influenced in part by complex load environment that may include fluid shear, substrate deformation, and hydrostatic pressure. *In vitro*, these distinct loading conditions have been shown to elicit different patterns of osteoblastic gene expression. For example, oscillatory fluid shear has been shown to inhibit TNF- α mediated NF- κ B activation [1], while static shear has been shown to activate the NF- κ B pathway [2]. The transcription factors NF- κ B, serum response factor (SRF), and c-fos are involved in many cell signaling pathways, including response to proliferative, apoptotic, and mechanical stress cues. The goal of this study was to examine changes in the temporal activation of these early transcription factors due to application of different magnitudes of oscillatory and pulse-static fluid shear.

METHODS Cell Culture

Mouse pre-osteoblastic (MC3T3-E1) cells were seeded @ 35,000 cells/cm² on covalently-modified, fibronectin-coated 38 x 57mm glass slides. Cast silicone rings 1.5cm thick were used to isolate cells to the laminar region of the slide and allowed rapid release for transfer to the shear chamber. Cells were grown in α -MEM + 10% FBS for 3 days and media changed to α -MEM with either 0% or 2% FBS 18-24 hours before shear loading.

Fluid Shear Loading

The shear system utilized was similar to that of Jacobs et al. [1]. Fluid shear was applied to cells in a polycarbonate parallel plate flow chamber via custom syringe pump. The system was used to apply pulsatile-static (shear pulses held at a constant amplitude) and oscillatory flow profiles to shear of magnitudes up to 2Pa. Oscillatory shear was applied sinusoidally at a frequency of 0.5Hz, while pulsatile-static shear was applied at 0.033Hz (15s forward, 15s reverse, with 0.2s transitions). Shear amplitudes were dependent on the depth of the chamber, at 10ml/min maximum flow rates, chambers of 0.26mm and 0.13mm resulted in maximum shear stresses of 0.5Pa

and 2.0Pa. Slides for culture controls were sealed & incubated with no flow, while slides for motion control were sealed in chambers briefly before they were placed back in the incubator in dishes with fresh media.

Immunofluorescence & Western Blotting

Cells were processed for immunofluorescence against SRF and the p65 subunit of NF- κ B and images were recorded and analyzed using the BioQuant Imaging System. Grayscale densities were recorded in the nuclei and cytoplasm of >50 cells in 6 images. Because NF- κ B is translocated from cytoplasm to nucleus upon activation, activation levels are reported as a ratio of nuclear to cytoplasmic densities.

Cell lysates were collected in lysis buffer and protein quantified. SDS-PAGE gels were transferred to nitrocellulose and probed for I κ B, NF- κ B (p65 subunit), SRF, and c-fos (Santa-Cruz). I κ B α is a protein which inhibits NF- κ B, and its degradation reflects NF- κ B activation. Densitometry was performed on a Bio-Rad Gel Densitometer. NF- κ B p65 are shown as gel loading controls and should not be shear dependent.

RESULTS

NF-KB Activation by Fluid Shear in 2%FBS

Both pulse-static and oscillatory shear induced NF- κ B activation in MC3T3 cells within 30 minutes as evidenced histologically by p65 translocation, though lower oscillatory shear had no effect (Figures 1 & 2). This induction was supported by I κ B degradation (Figure 3) in the same time course and the same conditions. Oscillatory flow at 2Pa resulted in a more rapid NF- κ B activation cycle, with a return to near baseline by 1hr. Shear mediated NF- κ B activation and I κ B degradation were not as significant in serum-free conditions.

Both controls did not activate NF- κ B as evidenced via histology or via Western blot.

c-fos Activation by Fluid Shear appears independent of srf

Both modes and amplitudes of shear in our experiment induced cfos expression within 45 minutes of shear exposure in 2% FBS media (Figures 3 & 4). However, this induction was not concomitant with a significant increase in srf expression via histology or Western blot (Figure 4). Similar to the results for NF- κ B, shear mediated c-fos activation was not as significant in serum-free conditions. In control slides, c-fos did appear to increase slightly after handling, but not to the level of shear mediated induction.

DISCUSSION

We have demonstrated that fluid shear can directly activate c-fos and NF- κ B, and that this activation is dependent on serum in the media. Our NF- κ B results support previous experiments using static shear [2], but differ from others utilizing oscillatory fluid shear [1]. These differences may be due to cell type, shear amplitude, or culture conditions. Pulse-static shear, which imposes a static shear on cells 99% of the time and has rapid transitions, had NF- κ B inductive effects at lower maximum shear stress and for a longer period than sinusoidal oscillatory shear.

Our c-fos results were consistent across shear type and amplitude, and these results are supported by work in previous static shear experiments [3]. Serum response factor is considered to be essential for c-fos induction, however it did not seem to play a role in our experiments. This may in part be due to the presence of 2% serum in the media, or a change in TCF phosphorylation which could stimulate the serum response element of the c-fos promoter if srf was abundant [4].

The activation of NF- κ B by oscillatory fluid flow in osteoblasts has not be previously reported, nor has the induction a srf independent c-fos response. These early response genes activate downstream genes which guide the process of bone adaptation, maintenance, and repair, such as OPN, COX-2, and osteocalcin. Understanding how osteoblasts respond via these early genes to varying mechanical loads *in vitro* increases our knowledge of how bone may respond to similar loads *in vivo*.

ACKNOWLEDGMENTS

This work was supported by NIH DE13014, AR46024, & T32-HD07505. We thank D Kayner, C Roehm, J Knowlton, L McCauley.











Figure 3. Shear-induced IKB degradation and c-fos activation



Figure 4. Shear-induced c-fos activation without srf induction

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