 MCB, vol.3, no.4, pp.219-220, 2006

Integrin Signaling and the Response of Osteocytes to Oscillatory Fluid Flow

J. B. Litzenberger [1, 2], P. Tummala [2] and C. R. Jacobs [1, 2]

1 Introduction

Osteocytes coordinate a cellular response to mechanical stimuli by directing effector cells to deposit or resorb bone [1]. Dynamic fluid flow has been shown to regulate bone cell metabolism, but the molecular mechanism through which osteocytes sense mechanical stimuli is unknown [2, 3]. Integrins, heterodimeric cell adhesion proteins, may serve as mechanosensitive molecules in bone.

The objective of this study is to analyze the role of integrin signaling in osteocyte mechanotransduction. We hypothesize that inhibited integrin signaling will decrease the response of osteocytes to oscillatory fluid flow, measured by gene expression and intracellular calcium mobilization. This study is novel in its quantification of the *in vitro* response of osteocytes with inhibited integrin signaling to dynamic flow.

2 Materials and Methods

Stably transfected cell lines of MLO-Y4 osteocytic cells were generated by transfecting parental cells either with an expression plasmid encoding a dominant negative form of the β1 integrin monomer (β1DN) or with the empty vector (vector controls). We exposed cells to oscillatory fluid flow (OFF) at peak shear stresses of 10 dynes/cm^2 and 1 Hz frequency. We used 10 μM Fura-2 AM to measure [Ca^{2+}] in each cell. Ca^{2+} response is defined as a transient increase in $[Ca^{2+}]_i$ of ≥4 times the maximum oscillation recorded during a 3 minute pre-flow period. For gene expression analysis, we lysed cells immediately after 2 hours of OFF. We performed Real Time RT-PCR using Taqman PCR Master Mix and 20X primers and probes for COX-2, OPG, and RANKL. Gene levels in each sample were normalized to the 18S housekeeping gene and were measured in triplicates. **Note:** We used the student *t* test to compare samples, and considered a $p < 0.05$ to be significant (*). Data are reported as mean ±SE.

[1]Stanford University, Bioengineering and Mechanica Engineering, Stanford, California, USA, 94305
[2]Veterans Affairs Palo Alto Health Care System, Palo Alto California, USA, 94304

3 Results

Application of OFF resulted in a significant increase in COX-2 mRNA levels in vector control cells (p = 0.001, N=4), but no significant change in β1DN cells (p=0.406, N=4) (**Fig. 1**). An average of 69% of the vector control cells responded to OFF with $[Ca^{2+}]_i$ mobilization, compared to 76% of the β1DN cells (p=0.361, N=9) (**Fig. 2**). RANKL/OPG mRNA levels decreased significantly after exposure to OFF in vector control cells (p=0.0007, N=7) but not in β1DN cells (p=0.247, N=7) (**Fig. 3**).

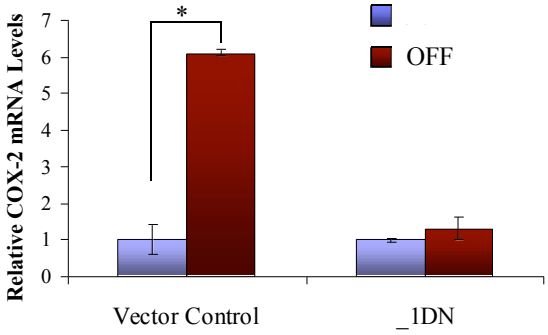

Figure 1 : COX-2 gene expression in cells with the application of oscillatory fluid flow (OFF) compared to controls (NF). * p<0.005.

4 Discussion

We found a significant increase in cyclooxygenase-2 (COX-2) transcription in response to OFF in control cells. Conversely, the flow induced increase in COX-2 gene expression was abrogated in β1DN cells (**Fig. 1**). *In vivo*, blocking production of COX-2, an enzyme associated with PGE_2 signaling in

bone cells [4], inhibits mechanically induced bone formation [5]. Our results indicate that inhibiting integrin signaling in osteocytic cells decreases the osteogenic response of these cells to mechanical stimuli. Intracellular Ca^{2+} release induced by OFF occurs independently of COX-2 transcription in bone cells [6,7]. We found no significant difference in the percentage of cells that responded to flow with intracellular calcium mobilization between control and β1DN cells (**Fig. 2**). Our data indicate that loading induced calcium release may occur independently of integrin signaling in osteocytes.

Osteoclast formation is governed by relative levels of receptor activator of nuclear factor kappa B (NF-κB) ligand (RANKL) and osteoprotegerin (OPG) in bone [8]. You and colleagues demonstrated that osteocyte-like cells exposed to OFF exhibit a decrease in the ratio of RANKL to OPG mRNA and have an inhibitory effect on osteoclast differentiation [9]. Our results indicate that inhibiting integrin signaling in osteocytic cells will reduce the cells' ability to mediate osteoclastogenesis.

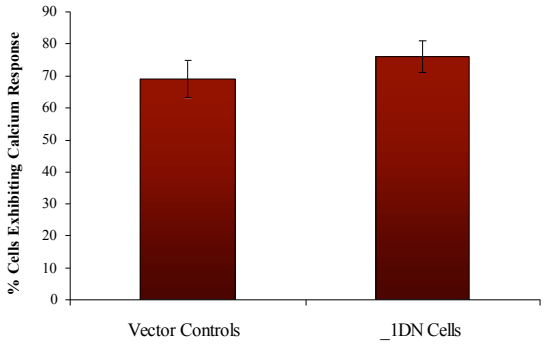

Figure 2 : Intracellular calcium mobilization in β1DN and control cells exposed to oscillatory fluid flow.

5 Conclusion

In summary, our data suggest that osteocytes may control the cellular response to mechanical stimuli by releasing both osteogenic and osteoclastic factors, which can thereby direct effector cells to deposit or resorb bone (**Figs. 1-3**). Inhibited integrin signaling results in abrogated COX-2 transcription and a reduced ratio of osteoclastic factors, but not decreased mobilization of intracellular calcium. Our results support the model that integrins are mechanosensitive molecules in bone, enabling osteocytes to sense mechanical stimuli and initiate intracellular signaling cascades.

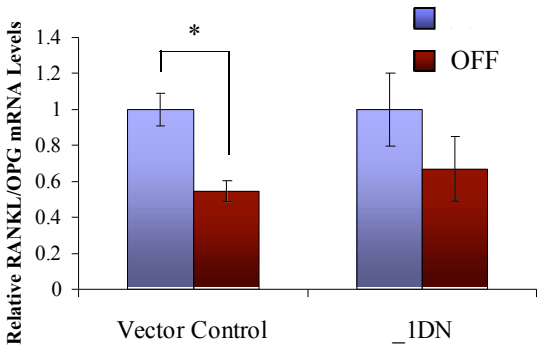

Figure 3 : RANKL/OPG gene expression with the application of oscillatory fluid flow (OFF) compared to controls (NF). * $p<0.005$.

Acknowledgement: Cells were donated by Dr. Ruth Globus (NASA Ames Research Center, CA). Research was supported by NIH Grant AR45989, NASA NAG2-1601, and a National Science Foundation Graduate Research Fellowship.

References

1. Burger, E.H. and Klein-Nulend. J. (1999).
2. Jacobs, C.R., *et al*. (1998).
3. You, J. *et al.*(2001).
4. Ponik, S.M. and Pavalko, F.M. (2004).
5. Forwood, M.R. (1996).
6. Saunders, M.M., *et al.* (2003).
7. Reilly, G.C., *et al.* (2003).
8. Boyle, W.J., *et al*. (2003).
9. You, L. *et al.* (2006) 52nd Ann ORS Conference.