The Effect of Mechanical Stimulation on Osteocyte Chemo-Sensitivity

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Introduction

Osteocytes are believed to be the mechanosensory cells that detect and respond to mechanical loading [1]. Mechanical loading results in oscillatory fluid flow (OFF) of interstitial fluid through the lacunae-canalicular networks and activates osteocytes to release prostaglandin E2 (PGE_2), a key molecule in the regulation of bone remodeling [2]. Osteocytes are also sensitive to chemical stimulation. In vitro studies have shown that serotonin [3] can rapidly induce PGE_2 release in osteocytes. MLO-Y4 osteocyte-like cells express the serotonin receptors and transporter mRNA expressions, and are capable of producing serotonin endogenously [3]. Exogenous serotonin increased osteocyte PGE_2 release by 3-fold in 1 hour. Nevertheless, the combined effects of mechanical and chemical stimulation on osteocytes have never been studied. Since osteocytes in bone can experience both types of stimulation simultaneously, it is unclear whether mechanical stimulation can influence the osteocyte sensitivity towards chemical stimulations. In this study, we hypothesize that mechanical stimulation can affect the sensitivity of osteocytes to chemical stimulations. Specifically, we investigate the effect of OFF on the osteocyte response to serotonin in releasing PGE_2.

Methods

Cell Culture: MLO-Y4 osteocyte-like cells (gift of Dr. Lynda Bonewald, UMKC) were cultured on type I rat tail collagen (BD Laboratory)-coated 100-mm tissue culture dishes in α-MEM (supplemented with 2.5% FBS, 2.5% CS, and 1% PS) and maintained at 37 ºC and 5% CO2 in a humidified incubator. Cell subculture was performed when the cells reached 70% confluence. For flow experiments, cells were seeded on collagen-coated glass slides (75mm × 38mm × 1mm) at 150,000 cells per slide 48 hr prior to flow experiment to ensure 80% confluence at the time of experiment.

Oscillatory Fluid Flow (OFF): Flow was driven by a Hamilton glass syringe in series with rigid walled tubing and a parallel plate flow chamber (38mm×10mm×0.25mm). The syringe was mounted in and driven by an electromechanical linear actuator. The flow rate was selected to produce a peak sinusoidal shear stress of 1 Pa at 1 Hz. Cells were exposed to 2 hr of OFF using fresh culture media. Control slides were incubated in the parallel flow chamber and were not subjected to OFF.

Chemical Treatment: To stimulate osteocytes with chemicals, cells were first washed with PBS, and media with or without 100 μM serotonin (Sigma) was applied to the cells using α-MEM with reduced serum (0.2% FBS, 0.2% CS, 1% PS) for 2 hr.

PGE_2 Release: Immediately following experiments, all the conditioned media were collected and centrifuged at 12,000 g. Supernatant PGE_2 levels were measured using Prostaglandin E2 EIA Kit (sensitivity: 50% B/B0: 50 pg/ml; 80% B/B0: 15 pg/ml) (Cayman Chemical, USA). PGE_2 levels of each experimental group were normalized to cell number.

Statistics: Student's t-test was used for two sample comparisons. ANOVA were used to compare observations from more than two groups, followed by Tukey post-hoc test (*p<0.05).

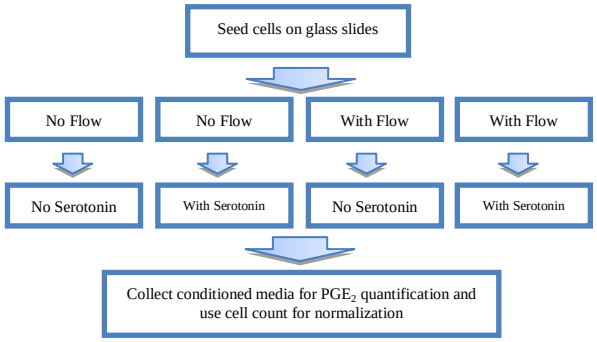


Figure 1: Experimental procedure for combined chemical and mechanical stimulation

Results

Two hours of serotonin treatment increased PGE_2 release by 3-fold in MLO-Y4 cells (Fig. 2).

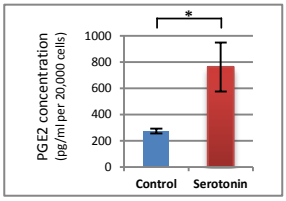
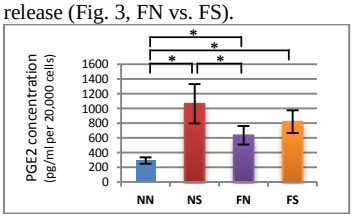


Fig. 2: Normalized PGE_2 concentration for chemical treatment in 6-well on MLO-Y4 cells, n=6 for all groups

Without mechanical stimulation, serotonin treatment increased osteocyte PGE_2 release by approximately 3-fold (Fig. 3, NN vs. NS). However, after MLO-Y4 osteocytes have been mechanically stimulated for 2 hr, the cells were no longer affected by serotonin treatment in PGE_2 release (Fig. 3, FN vs. FS).



NN: No flow, No serotonin
NS: No Flow, Serotonin only
FN: Flow only, No serotonin
FS: Flow and Serotonin

Fig. 3: PGE_2 concentration for combined chemical and mechanical treatment on MLO-Y4 cells according to Fig. 1, n=4 for all groups

Discussion

The present study explores the effect of mechanical stimulation on chemical sensitivity of osteocytes. Our first result (Fig. 2) is consistent with findings from other groups [3] that MLO-Y4 osteocytes respond to exogenous serotonin by increasing the release of PGE_2, which is a key molecule in the regulation of bone remodelling. Our second result (Fig. 3) is also consistent with previous findings [4] that OFF application leads to PGE_2 release in osteocytes. When MLO-Y4 osteocytes are subjected to mechanical stimulation first, as described in Fig. 1, the chemical stimulation by serotonin no longer had any effect on PGE_2 release. This suggests that pre-treating the osteocytes by mechanical stimulation can change their sensitivity towards chemical treatment.

In investigating the mechanism behind this interesting finding, one possibility is that mechanical stimulation altered part of the signalling pathway in serotonin-induced PGE_2 release. Since serotonin induced-PGE_2 depends on intracellular calcium mobilization and extracellular calcium levels [5], it is likely for calcium to play a role, and calcium may be a target for the OFF. On a broader scheme, gut-derived serotonin has recently been found to play a role in the lrp5-induced decrease in bone formation in osteoblasts, the current study adds a new angle to the finding by showing that osteocytes can also respond to serotonin and this affect can be modified by mechanical loading.

Significance

Osteoporosis is a musculoskeletal disease characterized by decreased bone mineral density and increased risk of bone fracture. Mechanical loading on bone has been shown to play a critical role in maintaining bone mineral density, thereby can be potentially used to treat and prevent osteoporosis. Here, we report the impact of mechanical loading on sensitivity of osteocytes to chemical stimuli.

Acknowledgements

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References

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