The Role of the Sphingosine-1-Phosphate Signaling Pathway in Osteocyte Mechanotransduction

Han, E S; Yu, X; Zhao, Y; Bolz, S; Lindington, D; Heximer, S; Simmons, C A; You, L

Division of Engineering Science, University of Toronto, ON, Canada, IBBME, University of Toronto, ON, Canada, Department of Physiology, University of Toronto, ON, Canada, Department of Mechanical and Industrial Engineering, University of Toronto, ON, Faculty of Dentistry, University of Toronto, ON, Canada

elizabeth.han@utoronto.ca

Introduction: The prevailing view of bone mechanobiology is that osteocytes are the cells responsible for detecting and responding to mechanical loading. Mechanical loading results in oscillatory flow of interstitial fluid through the lacuno-canalicular network and activation of cellular activities that regulate bone metabolism [1]. However, the exact signaling pathways involved are not well understood. Recent in vitro studies have shown that sphingosine-1-phosphate (S1P) signaling prominently regulates the myogenic response in resistance blood vessels in response to elevated transmural pressure [2]. S1P plays an important role in many biological processes, particularly cell proliferation [3], migration [4], and cytoskeletal rearrangement regulated by the Rho family [5]. S1P functions may also be related to the mobilization of calcium from intracellular stores independent of the IP3 mechanism [2]. The potential similarity between the mechanobiology of blood vessels and bones leads us to speculate that the S1P cascade might also be involved in osteocyte-regulated mechanotransduction (Figure 1). Therefore, the aim of this study was to confirm the presence of S1P signaling components in osteocytic cells. We also aim to test whether intracellular calcium mobilization, an early event in bone mechanotransduction, is affected by blockage of the S1P signaling pathway in flow-treated osteocytic cells.

Methods: Cell Culture: MLO-Y4 osteocyte-like cells (a gift from Dr. Lynda Bonewald, University of Missouri-Kansas City) were cultured 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. For RT-PCR, cells were cultured to confluency in 35 mm dishes. For calcium imaging experiments, cells were subcultured on UV transparent glass slides (3” x 1” x 0.96-1.06 mm) at 100,000 cells/slide. Fluid flow was applied 48 h after subculture such that the cells were 70% confluent at the time of experimentation. Cells were incubated at 37 ºC with Fura-2 AM (5 μM) (Invitrogen), a ratiometric fluorescent calcium indicator, for 30 minutes prior to fluid flow.

RT-PCR: Total RNA from fully confluent cells was extracted and purified using TRIzol (Gibco) for cDNA synthesis. Specific primer pairs (Table 1) for sphingosine kinase 1 and 2 (Sk1/Sk2), S1P phosphohydrolase 1, and S1P receptors S1P1, S1P2, and S1P3 were designed using published gene sequences (PubMed, NCBI Entrez Nucleotide Database), and RT-PCR was performed.

Oscillatory Fluid Flow (OFF): The flow media consisted of α-MEM, 1% FBS, and 1% CS. Flow was driven by a Hamilton glass syringe in series with rigid walled tubing and a parallel plate flow chamber, which was mounted on the stage of a fluorescent microscope (Nikon) equipped for computer image acquisition. 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. Images of fluorescent intensity were taken every 1 s for a 40 s preflow (baseline) period and a 2 min OFF period.

Intracellular Calcium Mobilization: Temporal profiles were determined for approximately 20 cells/slide. Each cell was classified as responding or not responding: a response was defined as a transient increase in the Fura-2 340 nm/380 nm ratio of at least 2 times the maximum oscillation recorded during the baseline period. Responsiveness was characterized by the fraction of responding cells.

Results: Expression of sphingosine kinases Sk1 and Sk2, S1P phosphohydrolase 1, and S1P receptors S1P1, S1P2, and S1P3 was detected in MLO-Y4 osteocyte-like cells (Figure 2). In the calcium imaging experiments, we observed a rapid transient increase in the Fura-2 340 nm/380 nm ratio, which is proportional to the cytosolic calcium concentration, immediately after the onset of OFF (Figure 3). A response to flow as defined was detected in 16% of cells.

Discussion: Our findings demonstrate that MLO-Y4 cells express all the components of the S1P signaling pathway. This suggests that the molecules necessary for the potential involvement of the cascade in osteocyte-regulated mechanotransduction are present. We also show in MLO-Y4 cells that there is a transient increase in mobilization of intracellular calcium in response to OFF. To further elucidate the speculated role of S1P in this early mechanotransductive event, we will induce overexpression and inhibition of sphingosine kinase, and determine the effect on calcium concentration. The effects of S1P on the expression of bone formation and resorption markers will also be quantified at the mRNA and protein levels. This is the first study of the role of the S1P signaling pathway in osteocytes. Our results will provide key information for understanding the cellular mechanism of bone mechanotransduction, which may lead to the development of a novel therapeutic strategy to treat bone diseases.


Acknowledgements: This research was supported by the National Sciences and Engineering Research Council (NSERC) Undergraduate Student Research Award (USRA), NSERC DG, and CFI.