PULSATING FLUID FLOW MODULATES GENE EXPRESSION OF PROTEINS INVOLVED IN WNT SIGNALING PATHWAYS IN OSTEOCYTES

Ana Santos1, Astrid D. Bakker1,2, Behrouz Zandieh-Doulabi2, Cornelis M. Semeins1, Jenneke Klein-Nulend1
1Dept Oral Cell Biology, ACTA-Vrije Universiteit and Universiteit van Amsterdam, Research Institute Move, Amsterdam, Netherlands; 2Dept Orthopaedic Surgery, Vrije Universiteit Medical Center, Research Institute Move, Amsterdam, Netherlands
a.santos@vumc.nl

Introduction: Bone is a living tissue that is able to adapt its mass and structure to mechanical loading. It is well accepted that osteocytes are the professional mechanosensory cells of bone. Strain-derived flow of interstitial fluid through the lacuno-canicular network activates cellular signaling transduction pathways in osteocytes that regulate bone metabolism [1,2]. It has been shown that proteins involved in the Wnt signaling pathway, i.e. β-catenin and lipoprotein receptor-related protein 5 (LRP5), are involved in the regulation of mechanical adaptation and mechano-sensitivity of bone [3,4]. Nevertheless it is still unclear whether mechano-sensitive osteocytes can modulate or activate the different Wnt signaling pathways in response to mechanical loading. Therefore the aim of our study was to assess whether a physiological mechanical stimulation, by means of pulsating fluid flow (PFF), affects gene expression of proteins involved in Wnt signaling in cultured osteocytes. Furthermore, we subjected cultured osteoblasts to PFF, in order to confirm the specificity of the osteocytic response to this stimulus.

Materials and Methods: MLO-Y4 osteocytes and MC3T3-E1 osteoblasts were submitted to 1 h of pulsating fluid flow (PFF, 0.7±0.3 Pa, 5 Hz) in a laminar flow chamber, and post-incubated without PFF for 0.5 to 3 h. Gene expression of proteins related to the Wnt canonical pathway (Wnt3, LRP5, LRP6, β-catenin, APC), the canonical Wnt signaling target gene c-jun, and the non-canonical pathway (Fzd6, Wnt5, Sfrp4), was studied using real time-PCR. Statistical analysis was performed using student t-test. Differences were considered significant if p<0.05.

Results: In MLO-Y4 osteocytes, 1 h of PFF followed by 1 h of post incubation (PI) without PFF, up-regulated gene expression of β-catenin by 1.3-fold, APC by 1.2-fold, and the Wnt target gene c-jun by 1.3-fold. Gene expression of Wnt3a, SFRP4, and c-jun was also up-regulated by 1.3, 1.5, and 1.5-fold respectively, at 3 h of PI (Figure 1). PFF down-regulated the expression of LRP5 and Fzd6, but did not affect gene expression of LRP6 in osteocytes. Wnt5 mRNA expression was detectable but not quantifiable.

In MC3T3-E1 osteoblasts, 1 h of PFF, followed by 0.5 h of PI down-regulated gene expression of Wnt5 by 1.7-fold. APC gene expression was down-regulated by 1.3-fold at 1 and 3 h of PI, LRP6, β-catenin, APC and c-jun gene expression were also down-regulated by 2.5, 20, 1.7 and 2-fold respectively (Figure 2). There was no effect of PFF on gene expression of Wnt3a, LRP5 and Fzd6.

Discussion: This is the first study showing that MLO-Y4 osteocytes respond to mechanical loading with modification of gene expression of proteins involved in the canonical Wnt signaling pathway. We observed up-regulation of mRNA expression of Wnt3, β-catenin, and the Wnt target gene c-jun by mechanical loading.

Osteocytes and osteoblasts exhibited different gene expression of proteins involved in Wnt signaling pathways after mechanical loading. This underscores the specificity of the mechano-response of osteocytes in terms of Wnt expression. This is not surprising considering the distinct roles of osteocytes as mechanosensors and osteoblasts as bone forming cells in bone physiology.

Wnts are involved in the regulation of bone mechanical adaptation, but it has been hitherto unknown which cells are involved in mediating the Wnt response to mechanical loading. Our results suggest that this Wnt response is, at least in part, orchestrated by osteocytes.

References:

Acknowledgements: The Dutch Program for Tissue Engineering (DPTE) supported the work of A. Santos (DPTE grant #V67744) and B. Zandieh-Doulabi (DPTE grant B676734). The Research Institute Move of the Vrije Universiteit supported the work of A.D. Bakker.