The Effect of Bone Anabolic Stimuli on Human Osteoblast to Osteocyte Transition

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ABSTRACT

Osteoporosis, a condition defined by a low bone mineral density (BMD) and associated with increased fracture risk, is associated with a decrease in both osteocyte (OY) density and viability. A great deal of evidence implicates OY as central to bone physiology and pathology (1). However, human OY biology in particular is poorly characterised. We previously showed that a variety of bone-acting factors induce a pro-anabolic or pro-catabolic response in human primary osteoblasts (Normal Human Bone-derived Cells, NHBC), concomitant with the acquisition of an OY-like phenotype (2-6). Bone mineralisation, the deposition of calcium and phosphate as calcium phosphate in the form of hydroxyapatite, occurs in lamellar bone concurrent with osteoblast to OY transition (7).

The first aim of the current study was to characterise the role of calcium, a common dietary supplement for the treatment of osteoporosis, in the transition of osteoblasts to OY, using human primary cell models. Secondly, low intensity pulsed ultrasound (LIPUS), an emerging therapy for osteoporosis and fracture repair, was also assessed for its effects on NHBC differentiation into OY. We hypothesised that each of these stimuli would exert a pro-anabolic effect on NHBC differentiation, promoting their transition to OY-like cells.

NHBC were cultured under conditions permissive for *in vitro* mineralisation, in the presence of a wide concentration range of Ca^{2+} (1.8 - 11.8 mM). Experiments were performed in the presence or absence of an inhibitor of the extracellular calcium sensing receptor (CaSR), NPS2390, as we hypothesised that these cells would 'sense' extracellular calcium through this receptor. NHBC tolerated even the highest concentration of Ca^{2+} used. Treatment with Ca^{2+} resulted in a striking dose- and time-dependent increase in *in vitro* mineralisation, associated with an increasing ratio of Ca:P, as determined by electron dispersive spectroscopy (EDS). Levels of mRNAs encoding the OY markers, SOST, E11 and dentin

matrix protein 1 (DMP1), were elevated in the mineralised cultures indicating promotion of osteoblast to OY transition. Gene expression was differentially regulated by Ca^{2+}. The expression of the osteoclast inhibitor, OPG, was dramatically enhanced by calcium. It was found that CaSR mRNA expression was rapidly lost from human trabecular bone *ex vivo* and is not expressed by NHBC. However, NHBC did express the related receptor, GPRC6A. Surprisingly, mineralisation was either unchanged or enhanced in the presence of the calcium sensing receptor inhibitor, NPS2390. Calcium-dependent mineralisation was reversed in the presence of phosphorylated MEPE-ASARM peptides. This study suggests that osteoblast to OY transition, and the concurrent mineralisation of the extracellular matrix, is sensitive to extracellular calcium independent of the canonical CaSR.

LIPUS is transmitted to target tissues as a low pressure acoustic wave (8), and has been shown to improve fracture healing (9-12). NHBC isolated from five donors were grown under conditions permissive for mineralisation and treated with a regimen of LIPUS at 1.5 mHz for 20 min daily for up to 7 days, either pre- or post-onset of mineralisation. The results showed a mild increase in the proliferation of cells in some cases in response to LIPUS treatment. Also, the expression of E11, a gene associated with osteoblast-OY transition, was increased. Cells from some donors responded to LIPUS by releasing measurable prostaglandin E2 (PGE_2), a response also associated with mechanical loading of bone and the effect of LIPUS in other models though there was no significant trend towards increased mineralisation. The results from this study suggest that LIPUS treatment may promote the differentiation of NHBC to a pre- or osteoid-OY-like phenotype. In summary, bone anabolic stimuli either in the form of calcium or LIPUS differentially affect the transition of osteoblasts to OY.

DECLARATION

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