Bisphosphonates modulate bone biomarkers in treated primary human osteoblasts

1)Giannasi C. 2)Niada S. 3)Brini AT.

University of Milan

Bisphosphonates (BPs) are synthetic analogues of pyrophosphate administered for the treatment of several diseases affecting the skeletal system, including osteoporosis, Paget disease, osteogenesis imperfecta and primary or secondary bone cancer. Although BPs are classified as anti-resorptive drugs, there is increasing evidence that they may play a role in bone formation as well. While the mechanism of action that these compounds exert on bone-resorbing osteoclasts has been fully elucidated, their direct effect on osteoblasts is still controversial (Fromigué et al., 2002; Açil et al., 2012). These molecules preferentially accumulate in areas with increased bone turnover (e.g. maxilla) and at the moment there are no univocal data on the BP concentrations at the different skeletal districts. With this in vitro study we investigated the direct effects of Alendronate (AL) and Zoledronate (ZL) on osteoblast viability and on some relevant bone metabolism markers such as ALP, SPARC, OPN and type I Collagen.

Human primary osteoblasts were derived from the femoral head of patients who underwent total hip replacement surgery at IRCCS Galeazzi Orthopaedic Institute. Single or repeated treatments with concentrations spanning from 10-15 to 10-5M of the nitrogen containing bisphosphonates AL and ZL were performed and cell viability was assessed at day 2, 5, 9 and 12 through Alamar Blue assay. We did not observe a significant influence by the BP treatments on osteoblast vitality and proliferation; only repeated treatments with 10-5M ZL significantly inhibit osteoblast vitality of about -36±9, -63±15 and -69±18% respect to untreated cells at day 5, 9 and 12, respectively. Analysis of alkaline phosphatase (ALP) activity at day 14 showed that low doses of AL (≤10-8M) and ZL (≤10-10M) seem to minimally enhance ALP activity, while higher concentrations of both drugs slightly inhibit it in a dose related trend. Then we evaluated the effects of 10-13, 10-10 and 10-7M of AL and ZL on the expression and secretion of bone markers. 7 days of treatment increase SPARC expression at all the tested concentrations with a more pronounced effect for ZL, while the expression of both OPN and type I Collagen seemed mildly reduced respect to control osteoblasts. Furthermore, we evaluated the effect of BPs on protein secretion and, interestingly, secreted OPN was increased by both drug treatments. Several bone biomarkers such as DKK-1, FGF-23, OC, OPN, OPG and SOST, together with inflammatory ones (IL-1β, IL-6, and TNF-α), will be soon analysed in supernatants of treated and untreated osteoblasts to determine the BP effect on bone cell communication. We confirm that therapeutic concentrations of AL and ZL do not affect osteoblast viability, while they differently modulate ALP, OPN, type I Collagen and SPARC expression. Taken together, our data suggest that high doses or accumulation of these drugs may alter bone turnover through a direct action on bone-forming cells.

Açil et al. J Craniomaxillofac Surg. 40, e229-35.

Fromigué et al. J Endocrinol Invest. 25, 539-46.