

Zoledronate treatment at subtoxic doses delays human primary osteoblasts differentiation

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Zoledronic acid (ZA) belongs to the family of bisphosphonates (BPs), largely used in the clinical practice for the treatment of bone diseases, often associated with jaw osteonecrosis onset. Their pharmacological action consists in the block of the osteoclast-mediated bone resorption along with indirect action on osteoblasts (2). The aim of this study was to check the effect of ZA at subtoxic dose on primary human osteoblasts (HOs) in terms of cell viability, apoptosis occurrence, and differentiation induction. HOs were treated choosing the limit concentration (10(-5) M) which does not induce toxic effects. Live/dead staining, flow cytometry to evaluate apoptotic markers, mitochondrial membrane potential assay, osteocalcin western blotting, gp38 RT-PCR, and collagen type I, PGE2, IL-6 ELISA tests were performed. The viability level between control and ZA-treated samples appears to be similar and no significant increase of apoptotic and necrotic cells in ZA-treated sample are evident. Bax expression and mitochondrial membrane potential were evaluated to establish if an early apoptotic pathway was triggered disclosing a higher protein expression in control sample and a good integrity of mitochondrial membrane in both experimental points. Type I collagen secretion and alkaline phosphatase activity are increased in ZA-treated sample, osteocalcin expression level is reduced in ZA-treated cells, whereas no modifications of gp38 mRNA level are evidenced. IL-6 secretion is lower in ZA-treated HOs with respect to control ones whereas no statistical differences are identified in PGE2 secretion level. These results highlight that ZA treatment at subtoxic dose is able to delay the osteoblastic differentiation process versus the osteocytic lineage, strengthening the pharmacological activity of the drug. Thus, the knowledge of ZA effects on osteoblasts allows to improve therapeutic protocols in order to strengthen drug pharmacological activity through a combined action on both osteoclasts and osteoblasts.

References

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Keywords

Zoledronic acid; Osteonecrosis of the jaw; Human osteoblasts; Apoptosis; Differentiation; PGE2; Collagen type I.