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Cytoprotective effects of melatonin on zoledronic acid-treated human osteoblasts

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ABSTRACT

Objective: To evaluate the cytoprotective effects of melatonin (MLT) on zoledronic acid (ZA)-treated human osteoblasts.
Methods: Human osteoblasts were exposed to ZA (1, 5, 10, 50, 100 and 300 μM) and MLT (1, 10, 50, 100 y 200 μM) for 24, 48 and 72 h of incubation, to evaluate their effects on cell viability.
Results: As ZA concentration increased, greater reductions in cell viability of human osteoblasts were induced whether at 24, 48 or 72 h incubation. At 24 h incubation with MLT, greatest cell viability was obtained when low dose of MLT was applied (without significant differences); 48 and 72 h incubation presented the greatest cell viability with the highest MLT concentrations (100 and 200 μM). MLT at concentrations of 100 and 200 μM would appear to have a certain cytoprotective effect on ZA-treated human osteoblasts with low concentrations of ZA (1 y 5 μM), whether at 24, 48 or 72 h; however, at ZA concentrations ≥10 μM the possible cytoprotective effects of MLT were low at 24 h incubation.
Conclusions: MLT has a cytoprotective effect on ZA-treated human osteoblasts and could represent a promising preventative alternative for patients at risk of bisphosphonate-related osteonecrosis of the jaw.

1. Introduction

Bisphosphonates (BPs) are chemically stable pyrophosphate composite analogues with a high affinity for hydroxyapatite, widely used for treating diseases that produce increases in bone resorption such as Paget's disease, and cancer-related bone diseases (both in patients with normal levels of serum calcium and in patients with tumor-induced hypocalcaemia). They are a basic treatment for osteoporosis and have also been used to treat some childhood skeletal diseases such as osteogenesis imperfecta (Aapro et al., 2008; Cheung and Glorieux, 2008). Although BPs are very effective in reducing bone loss, pain and several other skeletal clinical manifestations, they can induce adverse effects such as bisphosphonate-related osteonecrosis of the jaw (BRONJ) (Marx et al., 2005). BRONJ is defined as a condition characterized by non-healing exposed necrotic bone in the mandible or upper maxilla persisting for more than eight weeks in a patient who has taken or is currently taking a bisphosphonate, combined with an absence of head and neck radiation in the patient's history (Ruggiero et al., 2009). The American Association of Oral and Maxillofacial Surgeons (AAOMS) in 2014 recommended changing the nomenclature of BRONJ; they favored the term medication-related osteonecrosis of the jaw (MRONJ). The change was justified to accommodate the growing number of osteonecrosis cases involving the maxilla and mandible associated with other anti-resorptive and antiangiogenic therapies (Ruggiero et al., 2014).

Zoledronic acid (ZA) is a nitrogenous BP that has been developed exclusively for intravenous and not oral administration. The relatively long duration of its action is attributed to its high affinity for the center of farnesyl pyrophosphate synthetase (FPPS) action and

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its high capacity for attaching to bone mineral. However, ZA does not act exclusively on osteoclasts; recent research suggests that both osteocytes and osteoblasts may also be target cells for BPs in bone (Plotkin et al., 1999; Aguirre et al., 2006; Bonewald, 2007). Unlike BP's action on osteoclasts (whereby they induce apoptosis), in osteocytes the drugs inhibit apoptosis (Plotkin et al., 2006). ZA action on osteoblasts is controversial; while very low concentrations of BPs have been shown to stimulate osteoblasts *in vitro* (Rogers, 2003), high concentrations of ZA may reduce osteoblasts' migration capacity and cell viability (Koch et al., 2010; Walter et al., 2011).

Melatonin (MLT) (*N*-acetil-5-metoxitriptamina) is a neurohormone mainly synthesized and secreted by the pineal gland (mainly during the night-time part of the circadian cycle). Many of its effects differ from its primary neurohormonal functions and are due to its anti-inflammatory properties, its ability to act as an effective free-radical scavenger, and its capacity to stimulate several antioxidant enzymes (Czeniskiewiz-Guik et al., 2007). In relation to osteogenic activity, MLT has been shown to promote osteoblast maturation *in vitro* and prevent bone loss *in vitro* (Roth et al., 1999; Koyama et al., 2002; Satomura et al., 2007). In addition, it has been shown that physiological concentrations of melatonin boost alkaline phosphatase activity during the osteogenic differentiation processes of mesenchymal stem cells (Radio et al., 2006). Furthermore, it has recently been proved that MLT has a cytoprotective effect of MLT on human mesenchymal stem cells from periodontal ligament and bone marrow (Rodríguez-Lozano et al., 2015).

The aim of the present study was to evaluate the possible cytoprotective effects of MLT on ZA-treated human osteoblasts.

2. Material and methods

2.1. Cell line

The study used human osteoblast-like HOB-c cells (PromoCell, Heidelberg, Germany) cultured in an osteoblast-specific medium, Osteoblast Growth Medium (PromoCell, Heidelberg, Germany) composed of Dulbecco's Modified Eagle's Medium (DMEM) with 1% penicillin/streptomycin/neomycin (PSN), 1% L-glutamine and 10% fetal calf serum (FCS) at 37 °C, in an atmosphere of 95% oxygen and 5% CO_2. The medium was changed every other day.

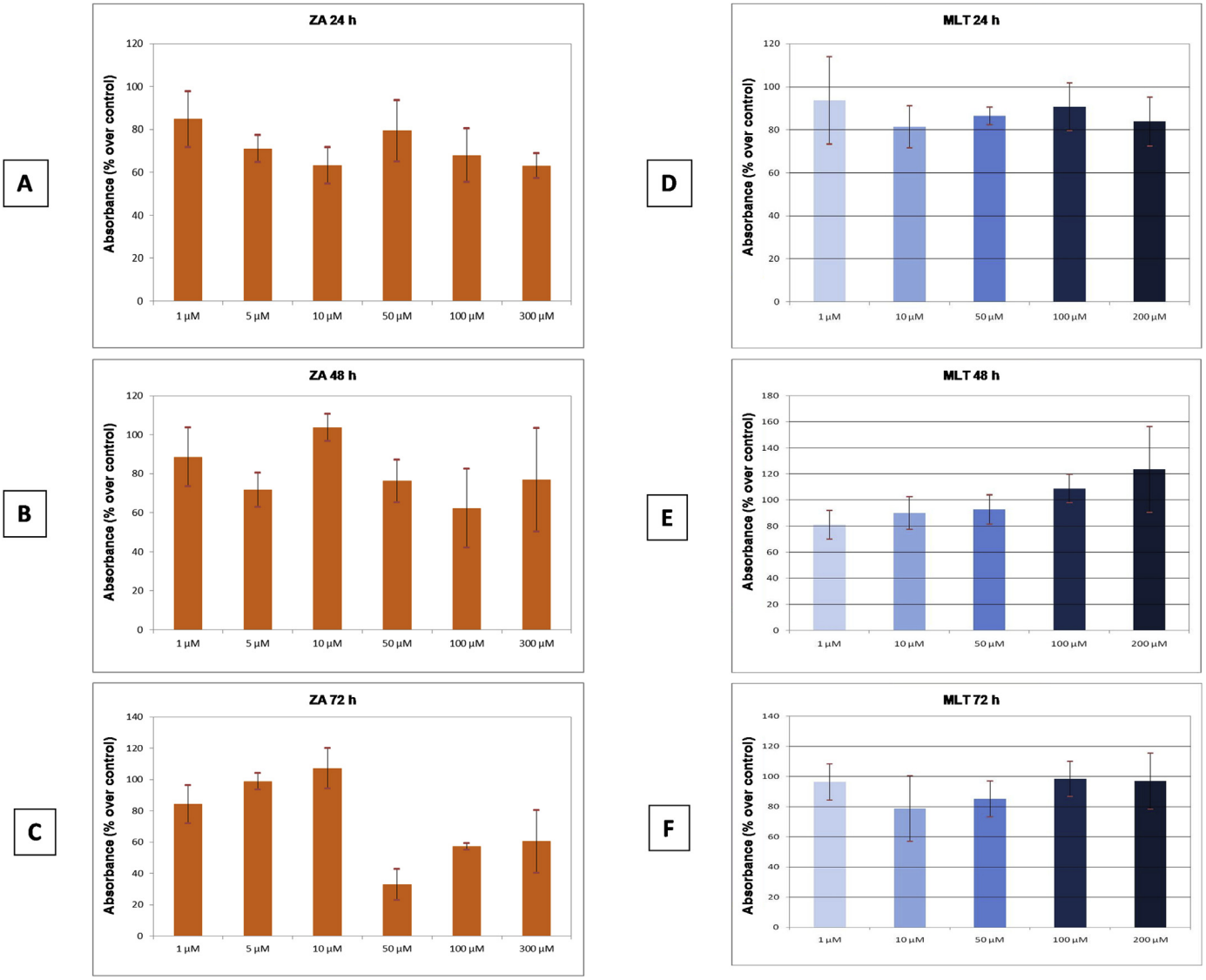

Fig. 1. Effects of ZA or MLT upon HOB-c cell viability at 24, 48 and 72 h of incubation (A: $p = 0.138$; B: $p = 0.102$; C: $p < 0.001$; D: $p = 0.446$; E: $p = 0.003$; F: $p = 0.149$).