MITOGENIC EFFECT OF BISPHOSPHONATES ON OSTEOBLASTIC CELLS

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Introduction

Bisphosphonates are a family of analogous synthetic pyrophosphates, which are effective inhibitors of bone resorption and are approved by the FDA to treat bone loss associated with osteoporosis and Paget's disease[1]. We have previously demonstrated that alendronate, a 2nd generation bisphosphonate, can effectively inhibit wear debris mediated osteolysis in a canine model[2]. Based on these convincing findings, the efficacy of alendronate in treating osteolysis is currently being evaluated in a multi-center clinical trial. We have also demonstrated in our canine model that in addition to inhibiting osteoclastic bone resorption, bisphosphonate therapy enhances net bone mass in implant porosities[3]. The significant increase in bone mass (>100%) is in excess of that which would be normally expected if the osteoclastic bone resorption was inhibited and normal osteoblast bone formation continued. We have thus hypothesized that bisphosphonates have an anabolic effect on osteoblasts. Thus, the purpose of this preliminary study was to investigate the effect of alendronate on the proliferation and activity of osteoblastic cells.

Materials and Methods

The effect of alendronate on two osteoblastic cell lines, SAOS-2, and MC-3T3-E1 was assessed. SAOS-2 cells (human osteosarcoma cells) were cultured in DMEM (Biowhittaker) supplemented with 10% FBS and 1% antibiotics (50U penicillin, 50ug streptomycin; GIBCO). MC-3T3-E1 clones (murine transformed osteoblast-like cells) were cultured in alpha MEM supplemented with 10% FBS, antibiotics, and L-glutamine (200mM)[4]. Cell cultures were performed at 95% humidity, 5% CO_2, and 37°C.

The effect of alendronate (Fosamax, Merck) over a wide concentration range (10^{-4} to 10^{-13} M), on the proliferation of osteoblasts was examined using the *MTT Colorimetric Assay*. Cells were plated at 25 x 10^4 cells/ml in 96-well plates. After an overnight culture, and media change, alendronate was added to different wells at different concentrations (10^{-4} to 10^{-13} M). One, 2, 3, 5, and 7 days after administering alendronate, cell proliferation was evaluated. Briefly, the tetrazolium salt MTT (Sigma, M-2128), was added at 50mg/ml in 50 ul and incubated for 2 h permitting mitochondrial conversion of MTT to formazan. The formazan was solubilized with 0.04N HCl isopropanol for 20 min, and quantitated in a microtiter plate reader using a 560nm filter. All experiments were conducted in triplicate and repeated.

Alkaline phosphatase enzyme activity in these two cell lines was determined using a commercially available assay (Sigma Diagnostics). In selecting the post-treatment time and concentration of alendronate, we were guided by the cell proliferation assays.

Osteoblastic cells were cultured in 12 well plates and grown to confluency. After treatment with alendronate over a range of concentrations, cultures were aspirated, washed with PBS, and the cells harvested by scraping. Cell fractions were lysed in water containing 25ul of 1% Triton X-100 and freeze-thawed twice to ensure lysis. Alkaline phosphatase activity in the cell fraction was measured by its conversion of a colorless p-nitrophenylphosphate to a colored p-nitrophenol. The color change was measured at 405nm and the amount of enzyme determined by comparing with a standard curve.

Results

A distinct dose-response effect was observed on treatment with alendronate for both osteoblastic cell lines. For the MC-3T3 cells, an inhibitory effect was observed at the highest dose of 10^{-4} M, and a mitogenic effect was observed between 10^{-8} M to 10^{-10}M. Alendronate was most effective at 10^{-8} M, and the peak effect was observed 48 h after treatment, which resulted in a 75% increase in cell proliferation (Fig. 1). Similar trends were observed with SAOS-2 cells, and the most effective concentration was also 10^{-8} M, while the peak stimulatory effect was observed 24 h after treatment resulting in a 61% increase over controls.

Alkaline phosphatase enzyme activity in the cell fraction increased after treatment with alendronate and trailed the cell proliferation by 24h. For MC-3T3 cells, while proliferation peaked 48h after treatment, alkaline phosphatase peaked 72h after treatment (Fig. 2) Similarly, for the SAOS-2 cells, while the maximal cell proliferation was observed at 24h after treatment, alkaline phosphatase activity increased after 48h and continued increases were detected at 72h after treatment.

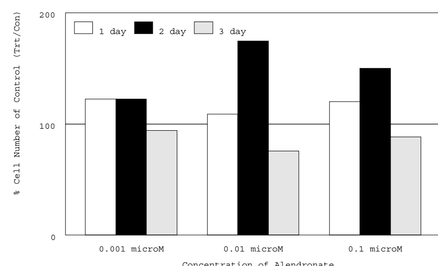

Fig. 1. Proliferation of MC3T3 E1 cells at different alendronate doses.

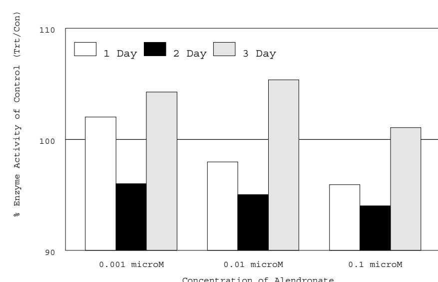

Fig. 2. Alkaline Phosphatase Activity in MC-3T3 E1 osteoblastic cells.

Discussion

While the ability of bisphosphonates to inhibit osteoclastic bone resorption has been well documented, their ability to enhance bone formation has not been proven. The findings in this report suggest that bisphosphonates can stimulate proliferation of osteoblastic cells as well as increase alkaline phosphatase levels. In follow-up studies, we will be studying the effect of alendronate and other bisphosphonates on the mineralization of the matrix.

In a clinical setting, oral therapy to enhance bone ingrowth into implant porosity provides a convenient and flexible therapeutic approach. Enhanced bone ingrowth around the femoral component can improve implant stability, and retard the migration of debris to the bone-implant interface. Further explorations will dissect this duality of bisphosphonate action, namely, inhibiting osteoclastic bone resorption and enhancing net bone formation.

References

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