Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 26 (2005) 6941–6949
Biomaterials
www.elsevier.com/locate/biomaterials

Effects of bisphosphonates on proliferation and osteoblast differentiation of human bone marrow stromal cells

Fabian von Knoch[a], Claude Jaquiery[b], Marc Kowalsky[a], Stefan Schaeren[b], Claude Alabre[a], Ivan Martin[b], Harry E. Rubash[a], Arun S. Shanbhag[a,*]

[a]*Biomaterials Laboratory, Department of Orthopaedic Surgery, Massachusetts General Hospital & Harvard Medical School, Boston, USA*
[b]*Departments of Surgery and of Research, University of Basel, Basel, Switzerland*

Received 25 January 2005; accepted 20 April 2005
Available online 11 July 2005

Abstract

Bisphosphonates are well known potent inhibitors of osteoclast activity and are widely used to treat metabolic bone diseases. Recent evidence from in vitro and in vivo studies indicates that bisphosphonates may additionally promote osteoblastic bone formation. In this study, we evaluated the effects of three FDA-approved and clinically utilized bisphosphonates, on the proliferation and osteogenic differentiation of human bone marrow stromal cells (BMSC).

BMSC were obtained from patients undergoing primary total hip arthroplasty for end-stage degenerative joint disease. Cells were treated with or without a bisphosphonate (alendronate, risedronate, or zoledronate) and analyzed over 21 days of culture. Cell proliferation was determined by direct cell counting. Osteogenic differentiation of BMSC was assessed with alkaline phosphatase bioassay and gene expression analyses using conventional RT-PCR as well as real-time quantitative RT-PCR.

All bisphosphonates tested enhanced the proliferation of BMSC after 7 and 14 days of culture. Steady-state mRNA levels of key genes involved in osteogenic differentiation such as bone morphogenetic protein-2 (BMP-2), bone sialoprotein-II, core-binding factor alpha subunit 1 (cbfa1) and type 1 collagen, were generally increased by bisphosphonate treatment in a type- and time-dependent manner. Gene expression levels varied among the different donors. Enhancement of osteogenic differentiation was most pronounced after 14 days of culture, particularly following zoledronate treatment ($p<0.05$ for BMP-2).

In conclusion, using a clinically relevant in vitro model we have demonstrated that bisphosphonates enhance proliferation of BMSC and initiate osteoblastic differentiation. When administered around joint replacements, bisphosphonates may potentially compensate for the deleterious effects of particulate wear debris at the bone–implant interface, by encouraging increased numbers of cells committed to the osteoblastic phenotype, and thus improve the longevity of joint replacements.

Keywords: Alendronate; Risedronate; Zoledronate; Bisphosphonates; Human bone marrow stromal cells; Bone morphogenetic protein; Total joint replacements

1. Introduction

Bisphosphonates are well-recognized inhibitors of osteoclastic activity and are widely used in the clinical treatment of various systemic metabolic bone diseases [1]. Current indications include Paget's disease, hypercalcemia of malignancy and post-menopausal osteoporosis [2–4]. Bisphosphonates are being investigated for the treatment of fibrous dysplasia [5], osteogenesis imperfecta [6], osteoarthritis [7] and rheumatoid arthritis [8].

Recent studies also indicate that bisphosphonates modulate wear-debris-induced inflammatory bone loss around total hip replacements (THR) through the inhibition of osteoclastic bone resorption. Using a canine THR model, Shanbhag et al. previously demonstrated that oral alendronate treatment can effectively

*Corresponding author. Tel.: +1 617 724 1923.
E-mail address: shanbhag@helix.mgh.harvard.edu (A.S. Shanbhag).

0142-9612/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2005.04.059

inhibit wear debris-mediated bone resorption [9]. These findings are supported by recent clinical studies suggesting that bisphosphonates also improve fixation and durability of total joint replacement components [10–13].

Although the primary action of bisphosphonates is the inhibition of osteoclastic bone resorption [1], there is increasing evidence that bisphosphonates also interact with osteoblasts. The pharmacological mechanism of action of the amino-bisphosphonates relies on interference with the mevalonate pathway through the inhibition of farnesyl pyrophosphate (FPP) synthase enzyme [14]. This causes a reduction in the levels of geranylgeranyl diphosphate (GGPP) required for the prenylation of guanosine triphosphate (GTP)-binding proteins such as Rab, Rac, Ras, Rho and Cdc42 [15,16]. Since these cytoskeletal regulators are essential for osteoclast activity and survival, bisphosphonates ultimately inhibit osteoclast formation and function. Statins, another class of drugs clinically used to suppress hepatic cholesterol synthesis, also act on the mevalonate pathway by blocking the more upstream 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Interestingly, statins increase osteoblastic bone formation both in vitro as well as in vivo, as first reported by Mundy and co-workers [17]. Concurrently it appears that bisphosphonates too may have anabolic effects on osteoblasts. Recent studies from several groups, including our own, indicate that bisphosphonates enhance proliferation and maturation of osteoblasts [18–20] and inhibit apoptosis [21]. These observations strongly support the suggestion that bisphosphonates have an anabolic effect on osteoblasts and subsequently promote bone formation.

The effects of bisphosphonates on early stages of osteoblastic differentiation are not yet well understood. Osteoblast progenitors derive primarily from among bone marrow stromal cells (BMSC). These pluripotent cells can differentiate into osteoblasts, adipocytes, fibroblasts and myocytes, and demonstrate a remarkable elasticity between the various differentiation pathways [22]. Since human bone marrow stromal cells are critically involved in maintaining the dynamic equilibrium of bone turnover, it is important to investigate how these cells respond to bisphosphonate treatment. Thus the objective of this study was to investigate the effects of three clinically used bisphosphonates (alendronate, risedronate, and zoledronate) on the proliferation and osteogenic differentiation of BMSC.

2. Materials and methods

2.1. Human bone marrow in-vitro model

Human bone marrow was obtained from the femora of 4 patients (mean age 68 ± 8 years, range 57–76) undergoing primary total hip arthroplasty (THA) for osteoarthritis. Subjects did not have other bone disorders such as rheumatoid arthritis or renal insufficiency. Bone marrow harvested during surgery was diluted with phosphate-buffered saline (PBS), and mononuclear BMSC were separated by density centrifugation on Percoll 1077 (Sigma, St. Louis, MO). BMSC were then cultured at a density of 400,000 cells/cm^2 in a 1:1 premix of Dulbecco's modified Eagle's medium and F-12 medium (Biowhittaker, Walkersville, MD) supplemented with 10% fetal bovine serum, 1% antibiotics/ antimycotics (100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B, L-glutamine (2 mM), 10 mM ß-glycero-phosphate and 0.1 mM L-ascorbic 2-phosphate at 37 °C with 95% humidity and 5% CO_2. Dexamethasone is known to be a critical media supplement for osteogenic differentiation of BMSC [23], and was intentionally withheld in this study as a standard media supplement, but used as a positive control to compare effects of bisphosphonates under suboptimal osteogenic cell culture conditions and established osteogenic media conditions.

BMSC were treated with three bisphosphonates diluted in sterile PBS: alendronate ([10^{-8} M] Fosamax, Merck, Rahway NY), risedronate ([10^{-8} M] Actonel, Proctor & Gamble, Cincinnati, OH), zoledronate ([10^{-8} M] Zometa, Novartis, Basel, Switzerland). Various types of controls including negative control (medium alone) and positive controls with addition of dexamethasone ([10^{-8} M], Sigma, St. Louis, MO) or 1,25-dihydroxy-cholecalciferol, vitamin D3 ([10^{-8} M], Sigma) were included. Culture media and drugs were replaced twice a week and experiments terminated 7, 14 and 21 days after culture initiation. Supernatants were collected for protein assay, cell lysates in TRIzol® (Gibco-BRL, Grand Island, NY) were collected for total RNA extraction and subsequent RT-PCR analyses. In order to ensure osteoblastic phenotype, BMSC cultures were stained for alkaline phosphatase in situ and evaluated by light microscopy.

2.2. Assessment of cell proliferation

BMSC were plated in 24-well plates and treated under different conditions as described above. After 7, 14, or 21 days of culture, cells were isolated from culture dishes by trypsinization, washed, and cell number and viability were determined with a hemocytometer using trypan blue dye exclusion test. Direct cell counts were performed in duplicate.

2.3. Alkaline phosphatase assay

Cells were plated in culture dishes (10 cm diameter) and treated as described above. Alkaline phosphatase (AP) activity after 7 and 14 days of culture was assayed utilizing the conversion of a colorless *p*-nitrophenyl phosphate to a colored *p*-nitrophenol (Sigma, St.Louis, MO). The color change was measured spectrometrically at 405 nm (Labsystems, Multiskan Multisoft), and the amount of enzyme released by the cells was quantified by comparison with a standard curve. AP levels were normalized to cell number at the end of the experiment. All experiments were conducted in duplicate, and repeated in BMSC cultures from three independent donors.