Biochemical Pharmacology 81 (2011) 1036–1042

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Dose-dependent differential effects of risedronate on gene expression in osteoblasts

J. Wang, P.H. Stern *

Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Feinberg School of Medicine. 303 E. Chicago Ave., Chicago, IL 60611, USA

ARTICLE INFO

Article history:
Received 5 January 2011
Accepted 31 January 2011
Available online 15 February 2011

Keywords:
Bisphosphonate
Risedronate
Osteoblast
Gene
Bone

ABSTRACT

Bisphosphonates have multiple effects on bone. Their actions on osteoclasts lead to inhibition of bone resorption, at least partially through apoptosis. Effects on osteoblasts vary, with modifications in the molecule and concentration both resulting in qualitatively different responses. To understand the mechanism of the differential effects of high and low bisphosphonate concentrations on osteoblast activity, we compared the effects of 10^{-8} M and 10^{-4} M risedronate on gene expression in UMR-106 rat osteoblastic cells. Two targeted arrays, an 84-gene signaling array and an 84-gene osteogeneic array were used. Gene expression was measured at 1 and 24 h. Although some genes were regulated similarly by low and high concentrations of the drug, there was also differential regulation. At 1 h, 11 genes (1 signaling and 10 osteogenesis) were solely regulated by the low concentration, and 7 genes (3 signaling, 4 osteogenesis) were solely regulated by the high concentration. At 24 h, 8 genes (3 signaling, 5 osteogenesis) were solely regulated by the low concentration and 30 genes (16 signaling and 14 osteogenesis) were solely regulated by the high concentration. Interestingly, the low, but not the high concentration of risedronate transiently and selectively upregulated several genes associated with cell differentiation. A number of genes related to apoptosis were regulated, and could be involved in effects of bisphosphonates to promote osteoblast apoptosis. Also, observed gene changes associated with decreased angiogenesis and decreased metastasis could, if they occur in other cell types, provide a basis for the effectiveness of bisphosphonates in the prevention of cancer metastases.

1. Introduction

Bisphosphonates are efficacious agents for the prevention and treatment of osteoporosis, for the antagonism of hypercalcemia, and for therapy of cancer metastases to bone. They are effective inhibitors of bone resorption, inhibiting osteoclastogenesis, osteoclast activity and osteoclast survival [1]. Although not all bisphosphonates affect resorption, those that do have essentially unidirectional effects, leading to the suppression of bone breakdown. In contrast, bisphosphonate effects on osteoblasts are more complex. Some bisphosphonates stimulate osteoblast proliferation, differentiation or survival, whereas others have inhibitory effects. Additionally, dose-dependent biphasic effects on proliferation or apoptosis have been documented for several bisphosphonates. Earlier studies showed that bisphosphonates (10^{-4}–10^{-5} M) decrease hFOB cell (fetal osteoblast cell) proliferation, but enhance differentiation and bone formation activities of the osteoblasts [2]. Bisphosphonates, at 10^{-5}–10^{-6} M can inhibit cell proliferation and induce apoptosis in UMR-106 osteoblastic cells [3]. Contrasting with this, other studies showed that low concentrations (10^{-6}–10^{-9} M) of bisphosphonates prevent apoptosis of osteoblasts [4,5]. Recent studies also indicate that bisphosphonates, including risedronate, over a broad concentration range (10^{-5}–10^{-12} M) enhance proliferation and differentiation of osteoblasts [6,7]. However, at a high concentration of 10^{-4} M, bisphosphonates decrease proliferation of MG-63 osteoblasts [7]. Both high and low bisphosphonate concentrations are potentially therapeutically relevant, since bisphosphonates concentrate in bone. High doses or prolonged treatment with bisphosphonates have now been associated with undesirable effects on bone, such as osteonecrosis of the jaw [8] and subtrochanteric fractures [9], which, although rare, can have devastating consequences. Inhibition of angiogenesis, suppressed bone remodeling, bone cell apoptosis, and collagen and mineralization abnormalities have been implicated in these bone side effects of bisphosphonates [10–14]. The more potent bisphosphonates are effective for inhibiting tumor cell metastases to bone [15,16].

* Corresponding author at: Department of Molecular Pharmacology and Biological Chemistry, S-215, Northwestern University Feinberg School of Medicine, 303 E Chicago Ave, Chicago, IL, 60611, USA. Tel.: +1 312 503 8290; fax: +1 312 503 5349.
E-mail addresses: j-wang4@northwestern.edu (J. Wang), p-stern@northwestern.edu (P.H. Stern).

0006-2952/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2011.01.025

The range of bone effects seen with bisphosphonates leads to the conclusion that there may be qualitative as well as quantitative differences elicited by different concentrations of bisphosphonates on osteoblasts. We have undertaken to investigate that possibility by comparing the effects on gene expression in rat osteosarcoma-derived UMR-106 osteoblastic cells of two widely different risedronate concentrations, a low concentration that is often used to simulate a therapeutic concentration, and the other representing a concentration that could be acquired by bone when exposed to high doses and which has antiproliferative effects on the osteoblastic cells. We have used a targeted osteogenic array to examine responses of the osteoblastic cells at the two concentrations, and also a targeted signaling array that could reveal pathways that lead to different responses. We have also used two time points, a 24 h time point, at which many responses should have been established, and a 1 h time point to identify dose-related differences that could represent possible initiating events.

2. Materials and methods

2.1. Cell culture

UMR-106 osteoblastic cells were purchased from American Type Culture Collection (Rockville, MD). The cells were cultured to confluence in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 100 U/ml penicillin G at 37 °C in a 5% CO_2 environment.

2.2. MTT assay

Cells were seeded at 18,000 cells per well in 96-well cell culture dishes in 0.2 ml of medium and allowed to attach overnight. They were then treated for 1 or 24 h with 0.2 ml of medium containing 10^{-4} M or 10^{-8} M risedronate (P&G Pharmaceuticals, Cincinnati, OH) or left untreated. At the end of the incubation period, 20 μl of 5 mg/ml MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) (Sigma, St. Louis, MO) in sterile phosphate-buffered saline was added to the medium in each well. After 1 h (for the 1 h treatment) or 4 h (for the 24 h treatment), the medium was removed and 200 μl dimethyl sulfoxide was added to each well and thoroughly triturated by pipetting up and down five times. Absorbance at 570 nm was measured with a Dynatech MR5000 plate reader.

2.3. ^{3}H-thymidine incorporation

For ^{3}H-thymidine incorporation, cells were seeded at 50,000 cells per well in 24-well cell culture dishes in 1 ml of medium and allowed to attach overnight. They were then treated for 1 or 24 h with 1 ml of medium containing 10^{-4} M or 10^{-8} M risedronate, or left untreated. For the final hour of the incubation, cells were labeled with 1 μCi/ml ^{3}H-thymidine (Amersham, Buckinghamshire, England), which was added in 5 μl of medium. They were then washed with 1 ml of medium. Medium was removed, and the plates placed on ice. The cells were incubated for 10 min with 0.5 ml 10% trichloroacetic acid, then washed 3 times with 0.5 ml 10% trichloroacetic acid and solubilized by incubation for 2 h at room temperature with 0.5 ml 0.5 N KOH. 1 N HCl was added to neutralize, and the samples were counted in Ultima Gold scintillation solution (PerkinElmer, Boston) with a Beckman LS 6500 scintillation counter.

2.4. Gene expression profiling using real-time PCR gene array

Cells were seeded at 500,000 cells per well in six-well cell culture dishes in 2 ml of medium and allowed to attach overnight.

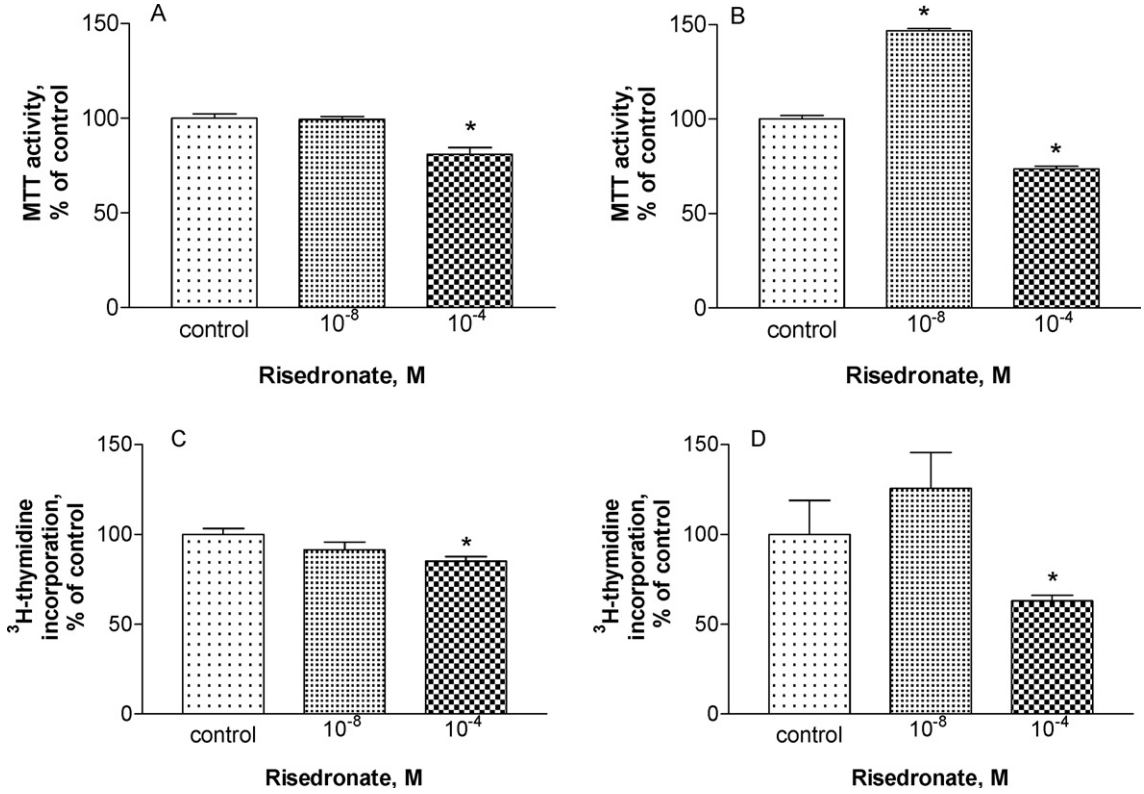

Fig. 1. MTT activity (A and B) and ^{3}H-thymidine incorporation (C and D) elicited by treatment of UMR-106 cells for 1 h (A and C) or 24 h (B and D) with 10^{-8} or 10^{-4} M risedronate. For MTT assays, $N = 6$, for ^{3}H-thymidine incorporation, $N = 3$. Data were analyzed by analysis of variance and significance determined by and Dunnett's test. *$P < 0.05$ vs. untreated control.