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Vitamin D antagonist, TEI-9647, inhibits osteoclast formation induced by 1α,25-dihydroxyvitamin D_3 from pagetic bone marrow cells☆

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Abstract

(23*S*)-25-Dehydro-1α-hydroxyvitamin D_3-26,23-lactone (TEI-9647) functions an antagonist of the 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) nuclear receptor (VDR)-mediated differentiation of human leukemia (HL-60) cells [J. Biol. Chem. 274 (1999) 16392]. We examined the effect of vitamin D antagonist, TEI-9647, on osteoclast formation induced by 1α,25-$(OH)_2D_3$ from bone marrow cells of patients with Paget's disease. TEI-9647 itself never induced osteoclast formation even at 10^{-6} M, but dose-dependently (10^{-10} to 10^{-6} M) inhibited osteoclast formation induced by physiologic concentrations of 1α,25-$(OH)_2D_3$ (41 pg/ml, 10^{-10} M) from bone marrow cells of patients with Paget's disease. At the same time, 10^{-8} M of TEI-9647 alone did not cause 1α,25-$(OH)_2D_3$ dependent gene expression, but almost completely suppressed TAF_{II}-17, a potential coactivator of VDR and 25-hydroxyvitamin D_3-24-hydroxylase (25-OH-D_3-24-hydroxylase) gene expression induced by 10^{-10} M 1α,25-$(OH)_2D_3$ in bone marrow cells of patients with Paget's disease. Moreover, TEI-9647 dose-dependently inhibited bone resorption induced by 10^{-9} M 1α,25-$(OH)_2D_3$ by osteoclasts produced by RANKL and M-CSF treatment of measles virus nucleocapsid gene transduced bone marrow cells. These results suggest that TEI-9647 acts directly on osteoclast precursors and osteoclasts, and that TEI-9647 may be a novel agent to suppress the excessive bone resorption and osteoclast formation in patients with Paget's disease.

Keywords: Vitamin D antagonist; Osteoclast; Bone resorption; Coactivator; Vitamin D receptor; Paget's disease; Measles virus

1. Introduction

Although Paget's disease of the bone is the most flagrant example of disordered bone remodeling, little is known about its pathogenesis. Recently, we reported that measles virus infection may play a major role in pathogenesis of Paget's disease including contributing to the hyper-responsivity of osteoclast precursors to 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) through increasing expression of TAF_{II}-17, a potential coactivator of vitamin D receptor (VDR) in pagetic osteoclast precursors [1–3]. Pagetic osteoclast precursors require at least 10 to 100 times less 1α,25-$(OH)_2D_3$ to form osteoclasts than that required for normal bone marrow cells. These results suggest that in Paget's disease bone resorption might be enhanced by physiologic levels of 1α,25-$(OH)_2D_3$. If this is correct, vitamin D antagonists that suppresses the function of 1α,25-$(OH)_2D_3$ could be potential agents to inhibit the enhanced bone resorption in patients with Paget's disease. Here we report that vitamin D antagonist, TEI-9647, inhibited osteoclat formation and bone resorption induced by 1α,25-$(OH)_2D_3$ by osteoclasts derived from pagetic bone marrow cells. At the same time, TEI-9647 also suppressed TAF_{II}-17 gene expression induced by 1α,25-$(OH)_2D_3$ in pagetic bone marrow cells.

2. Materials and methods

2.1. Subjects and cell preparation

The Institutional Review Board of the University of Pittsburgh approved these studies. Bone marrow cells were aspi-

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rated under 2% xylocaine anesthesia from the iliac crest of healthy normal volunteers or patients with Paget's disease into heparinized α-MEM containing 5% FBS. Bone marrow mononuclear cells were then isolated by separation on Hypaque–Ficoll gradients as described previously [4].

2.2. Measles virus nucleocapsid (MVNP) gene transduction of human bone marrow cells

Human bone marrow mononuclear cells were cultivated for 2 days in α-MEM containing 10% FBS that contained 10 ng/ml each of IL-3, IL-6 and stem cell factor (SCF) (Immunex Research and Development Corporation, Seattle, WA). The bone marrow cells were then cultured for an additional 48 h at 37 °C in a humidified atmosphere of 5% CO_2–air at a density of 1 to 2 × 10^5 cells/ml with supernatant (10% (v/v)) containing MVNP vector. Cultures were supplemented with 4 μg/ml of polybrene, 20 ng/ml of IL-3, 50 ng/ml of IL-6 and 100 ng/ml of SCF as described previously [2]. MVNP transduced cells were suspended 10^6 cells/ml in α-MEM containing 1.2% methylcellulose, 30% FBS, 1% deionized BSA, and 100 pg/ml recombinant human GM-CSF (Immunex Research and Development Corporation, Seattle, WA) with 500 μg/ml of G418. Transduced cells were plated in a volume of 1 ml in 35 mm culture dishes and incubated at 37 °C in a humidified atmosphere of 5% CO_2–air for 7 days. G418-resistant colonies were individually collected, using finally drawn pipettes, for use in all osteoclast formation assay as a MVNP transduced CFU-GM cells.

2.3. Osteoclast formation induced by vitamin D_3 analogues

Normal bone marrow mononuclear cells (10^6 cells/ml) were dispersed into a α-MEM containing 20% horse serum, and were seeded in 96-well multi-plates at 100 μl/well. A 10^{-8} M of 1α,25-$(OH)_2D_3$, 10^{-11} M to 10^{-6} M of TEI-9647, or a combination of them was each added into a well. Half of the media was replaced two times a week, and the culture was continued for 3 weeks at 37 °C in an incubator of 5% CO_2–air. After the culture, the formed osteoclasts were fixed with 2% formaldehyde and tested for cross-reactivity with monoclonal antibody 23C6 as described previously [2]. A cell that was positive to 23C6 antibody and had 3 or more nuclei was scored an osteoclast using an inverted microscope. In the case of MVNP transduced CFU-GM cells or pagetic bone marrow cells, we used a concentration of 10^{-9} M of 1α,25-$(OH)_2D_3$ instead of 10^{-8} M of 1α,25-$(OH)_2D_3$ in normal bone marrow cells.

2.4. Osteoclastic bone resorption on dentin slices

Normal bone marrow cells (2 × 10^6 cells/ml) were dispersed into α-MEM medium containing 20% horse serum, and were seeded on dentin slices (Wako Pure Chemical Industries, Osaka, Japan) in 96-well multi-plates at 100 μl/well. Fifty nanogram per milliliter RANKL and 50 ng/ml M-CSF (Wako Pure, Osaka, Japan) were added into a well as a stimulator of osteoclast formation. Half of the media containing 20% horse serum, RANKL and M-CSF was replaced two times a week, and culture was continued for 3 weeks at 37 °C in an incubator of 5% CO_2–air. After 3 weeks, RANKL and M-CSF was removed from the media for 3 days, and then the osteoclasts that had formed were activated by the treatment with 10^{-8} M 1α,25-$(OH)_2D_3$, or 10^{-9} to 10^{-6} M of TEI-9647, or a combination of both for 10 days. After 10 days, osteoclasts on the dentin slices were digested with 0.25% trypsin at 37 °C for overnight, and then resorption lacunae were stained with hematoxylin. Pit area was quantified by image analysis.

2.5. Gene expression induced by 1α,25-$(OH)_2D_3$ in pagetic bone marrow cells

Bone marrow cells (5 × 10^5 cells/ml) from patients with Paget's disease were cultured in α-MEM containing 10% FBS for 12 h with 10^{-10} M1α,25-$(OH)_2D_3$, or 10^{-8} M TEI-9647, or both agents. Total RNA extraction and RT-PCR were carried out as described previously [2]. The gene specific primers for TAF_{II}-17 (GenBank accession number U57693) were 5′-CATGCCATGGCTATGAACCAGTTT GGCCCCTCA-3′ (sense) and 5′-ATACTGCAGTTATTT-CTTGGTTGTTTTCCG-3′ (antisense). The gene specific primers for 25-OH-D_3-24-hydroxylase (GenBank accession number L13286) were 5′-ATTACCTGAGAATCAGAGG-CCACG-3′ (sense) and 5′-GCCAAATGCAGTTTAA GCTCTGCT-3′ (antisense). The conditions for amplification were as follows: a 5 min initiation step at 94 °C; 35 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min; and finally an extension step at 72 °C for 7 min. PCR products were separated by 2% agarose gel electrophoresis and were revealed with ethidium bromide staining under ultraviolet light. The relative amounts of TAF_{II}-17 mRNA and 25-OH-D_3-24-hydroxylase mRNA were determined by densitometry and compared with β-actin mRNA expression levels.

2.6. Measurement of serum concentrations of vitamin D metabolites

Serum samples were collected from nine patients with Paget's disease and 10 age-matched healthy normal volunteers (3–5 ml per subject). The serum concentrations of vitamin D metabolites were measured as described previously [5].

3. Results

We previously reported that osteoclast formation from bone marrow cells from patients with Paget's disease

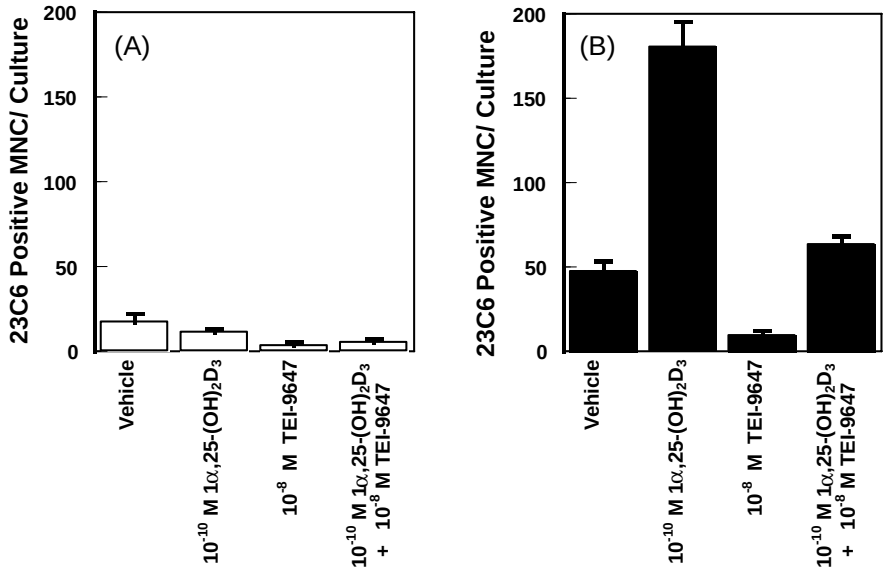

Fig. 1. Effect of TEI-9647 on osteoclast formation induced by physiologic concentration of 1α,25-$(OH)_2D_3$ from bone marrow cells of normal volunteers (A) and patients with Paget's disease (B).

could be induced by extremely low concentrations of 1α,25-$(OH)_2D_3$ that are at least 1/10 to 1/100 below that required for osteoclast formation from normal bone marrow cells. Therefore, we determined the serum concentrations of vitamin D metabolites in patients with Paget's disease. The serum concentrations of vitamin D metabolites in patients with Paget's disease were almost the same compared to age matched normal volunteers; 25-OH-D, 40.5 ± 11.1 ng/ml

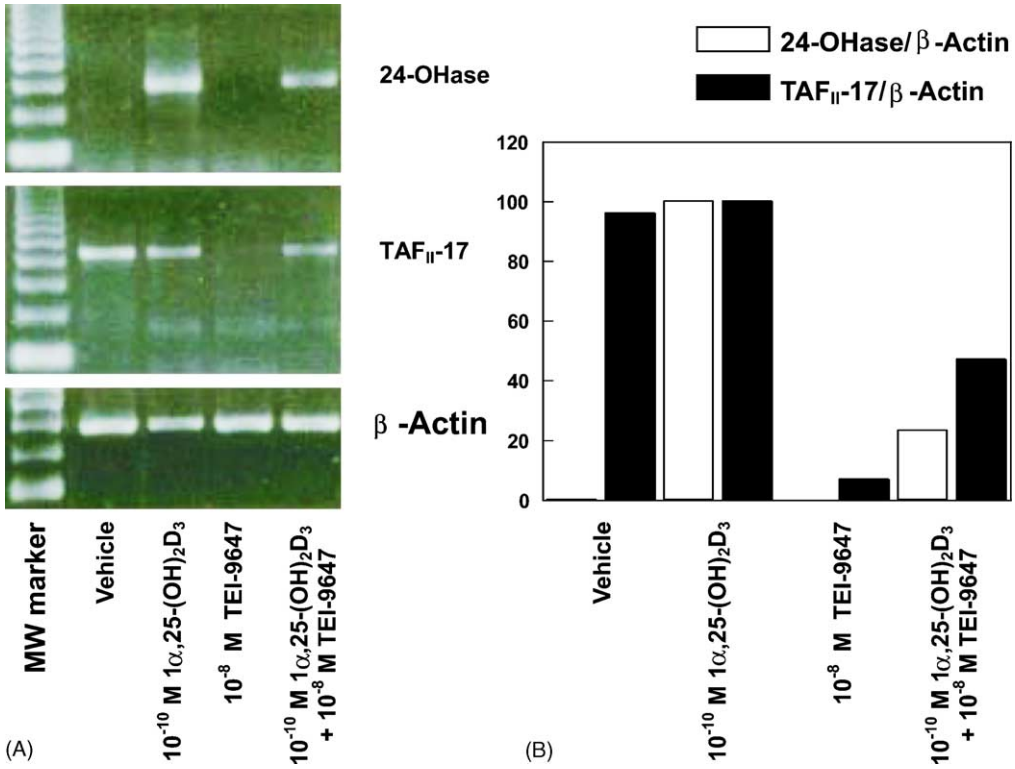

Fig. 2. Effect of TEI-9647 on the TAF_{II}-17 and 25-OH-D_3-24-hydroxylase gene expression induced by 10^{-10} M of 1α,25-$(OH)_2D_3$ in bone marrow cells from patients with Paget's disease. (A) Pagetic bone marrow cells were incubated with 10^{-8} M of TEI-9647 in the absence or presence of 10^{-10} M of 1α,25-$(OH)_2D_3$ for 12 h. Total RNA was extracted and RT-PCR of TAF_{II}-17, 25-OH-D_3-24-hydroxylase or β-actin was done as described in Materials and Methods. (B) Densitometric scanning of the lanes of (A).

versus 39.3 ± 9.5 ng/ml; 24,25-$(OH)_2D$, 2.64 ± 1.57 ng/ml versus 2.39 ± 1.09 ng/ml; 1α,25-$(OH)_2D$, 41.0 ± 9.1 pg/ml versus 38.8 ± 12.0 pg/ml. On the other hand, serum alkaline phosphatase in patients with Paget's disease was markedly increased compared to normal volunteers; 399 ± 61 IU/l versus 177 ± 12 IU/l. Serum concentrations of 1α,25-$(OH)_2D$ were 41 pg/ml (10^{-10} M). A 10^{-10} M of 1α,25-$(OH)_2D_3$ minimally induced osteoclast formation from normal bone marrow cells but it markedly induced osteoclast formation from bone marrow cells in patients with Paget's disease (Fig. 1). TEI-9647 alone never induced osteoclast formation, but 10^{-8} M of TEI-9647 markedly inhibited osteoclast formation induced by 10^{-10} M of 1α,25-$(OH)_2D_3$ from bone marrow cells of patients with Paget's disease (Fig. 1). Dose-response experiments showed that 10^{-7} M of TEI-9647 almost completely inhibited osteoclast formation induced by 10^{-10} M of 1α,25-$(OH)_2D_3$.

Next we examined the action of TEI-9647 on bone resorption induced by 10^{-9} M 1α,25-$(OH)_2D_3$ by osteoclasts derived from pagetic bone marrow cells. TEI-9647 alone never induced bone resorption even at 10^{-6} M, but dose-dependently (10^{-9} M to 10^{-6} M) inhibited bone resorption induced by 10^{-9} M of 1α,25-$(OH)_2D_3$.

Fig. 2 showed that 10^{-8} M of TEI-9647 markedly inhibited TAF_{II}-17 and 25-OH-D_3-24-hydroxylase gene expression induced by 10^{-10} M of 1α,25-$(OH)_2D_3$ in bone marrow cells from patients with Paget's disease. In the case of MVNP transduced CFU-GM cells, similar results were obtained.

4. Discussions

We previously reported that both measles virus infection and the presence of 1α,25-$(OH)_2D_3$ may be involved in the pathogenesis of Paget's disease [1–3,6]. First measles virus infection to osteoclast precursors caused the expression of TAF_{II}-17, a potential coactivator of vitamin D receptor (VDR) [N. Kurihara, Personal communication], and the expression of TAF_{II}-17 gene enhanced the sensitivity of osteoclast precursors to 1α,25-$(OH)_2D_3$ [3]. Next with regard to serum concentration of 1α,25-$(OH)_2D_3$ in patients with Paget's disease they have 41 pg/ml, 10^{-10} M, the same level as normal volunteers have, which is enough to induce osteoclast formation by pagetic but not normal bone marrow cells and stimulate their bone resorption.

Our vitamin D antagonist, TEI-9647, removed the hyper-responsivity to 1α,25-$(OH)_2D_3$ by inhibiting the TAF_{II}-17 gene expression induced by 1α,25-$(OH)_2D_3$, and suppressed osteoclast formation and osteoclastic bone resorption. These results suggest that TEI-9647 acts directly on osteoclast precursors and osteoclasts, and that TEI-9647 may possibly be novel agent to suppress the excessive bone resorption and osteoclast formation in patients with Paget's disease.

References

[1] C. Menaa, J. Barsony, S.V. Reddy, J. Cornish, T. Cundy, G.D. Roodman, 1,25-Dihydroxyvitamin D_3 hypersensitivity of osteoclast precursors from patients with Paget's disease, J. Bone Miner. Res. 15 (2000) 228–236.

[2] N. Kurihara, S.V. Reddy, C. Menaa, D. Anderson, G.D. Roodman, Osteoclasts expressing the measles virus nucleocapsid gene display a pagetic phenotype, J. Clin. Invest. 105 (2000) 607–614.

[3] N. Kurihara, S.V. Reddy, J. Cornish, G.D. Roodman, Role of TAF_{II}-17 in the enhanced 1,25-dihydroxyvitamin D_3 responsivity of osteoclast (OCL) precursors from Paget's disease, J. Bone Miner. Res. 17 (suppl 1) (2002) S380–S389.

[4] T. Kukita, C. Chenu, L.M. McManus, G.R. Mundy, G.D. Roodman, A typical multinucleated cells form in long-term marrow cultures from patients with Paget's disease, J. Clin. Invest. 85 (1990) 1280–1286.

[5] S. Ishizuka, T. Naruchi, Y. Hashimoto, H. Orimo, Radioreceptor assay for 1α,24(R)25-trihydroxyvitamin D_3 in human serum, J. Nutr. Sci. Vitam. 27 (1981) 71–75.

[6] S.V. Reddy, N. Kurihara, C. Menaa, G. Landucci, D. Forthal, B.A. Koop, J.J. Windle, G.D. Roodman, Osteoclasts formed by measles virus-infected osteoclast precursors from hCD46 transgenic mice express characteristics of pagetic osteoclasts, Endocrinology 142 (2001) 2898–2905.