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Inhibitory Effects of 1′,2′-Dihydrorotenone on Osteoclast Differentiation and Bone Resorption In Vitro and In Vivo

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Abstract : It is important to identify therapeutic compounds with no adverse effects for use in the chemotherapy of patients with bone-related diseases. The aim of this study was to identify a new compound that inhibits osteoclast differentiation and bone resorption. Herein, we examined the effects of 1′,2′-dihydrorotenone on osteoclast differentiation and bone resorption in vitro and in vivo. 1′,2′-dihydrorotenone inhibited receptor activator of NF-κB ligand (RANKL)-induced osteoclast differentiation of cultured bone marrow macrophages (BMMs) in a dose-dependent manner. However, 1′,2′-dihydrorotenone did not exert cytotoxic effect on BMMs. 1′,2′-dihydrorotenone suppressed the expression of c-fos and NFATc1 as well as osteoclast-specific genes in BMMs treated with RANKL. Treatment with RANKL inhibited the expression of inhibitors of differentiation/DNA binding (Id)1, 2, and 3; however, in the presence of 1′,2′-dihydrorotenone, RANKL did not suppress the expression of Id1, 2, and 3. Furthermore, 1′,2′-dihydrorotenone inhibited bone resorption and considerably attenuated the erosion of trabecular bone induced by lipopolysaccharide treatment. Taken together, these results suggest that 1′,2′-dihydrorotenone has the potential to be applied in therapies for bone-related diseases.

Keywords : Osteoclast, RANKL, 1′,2′-dihydrorotenone, Resorption

Introduction

It is important to maintain the balance between the activities of osteoclast and osteoblast formation to maintain bone mass. Deviation of this balance toward osteoclast formation will increase bone resorption rather than bone formation and lead to pathological bone erosion and osteoporosis (Rodan and Martin 2000, Teitelbaum 2003). Decrease in bone mass resulting from dysregulated bone remodeling can be caused by systemic alterations, such as estrogen deficiency and local inflammation, which often leads to bone diseases such as rheumatoid arthritis and periodontitis (Boyle et al. 2003, Walsh et al. 2006). Osteoclasts are bone resorbing cells that differentiate from monocyte-macrophage lineage of hemopoietic cells and play a critical role in bone erosion in vivo. Osteoclasts differentiate to tartrate-resistant acid phosphatase (TRAP)-positive cells and fuse to form multinucleated cells; this process plays a critical

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role in bone remodeling (Suda et al. 1999, Walsh et al. 2006).

Receptor activator of NF-κB ligand (RANKL), which belongs to the tumor necrosis factor (TNF) superfamily, has been shown to induce osteoclast differentiation, survival, and function. RANKL binds to the receptor RANK and recruits TNF receptor-associated factor (TRAF) 6 to the cytoplasmic tail of RANK on the surface of osteoclast precursors (Kong et al. 1999, Suda et al. 1999, Kim et al. 2000). The recruitment of TRAF6 with RANK activates several intracellular signaling pathways, leading to osteoclast differentiation (Wong et al. 1998, Walsh and Choi 2003). Various transcription factors are required for the differentiation of osteoclast precursors to multinucleated osteoclasts (Takayanagi 2007). In particular, c-Fos, an immediate-early gene, plays an important role in the initiation of osteoclast differentiation through the expression of nuclear factor of activated T cells (NFAT) c1, which induces osteoclast-specific genes such as TRAP, calcitonin receptor, and osteoclast-associated receptor (OSCAR) (Takayanagi et al. 2002, Kim et al. 2005, Takayanagi 2007). Ectopic expression of NFATc1 in bone marrow macrophages (BMMs) is sufficient to induce osteoclast differentiation (Takayanagi et al. 2002).

It was recently reported that RANKL inhibits the expression of inhibitors of differentiation/DNA binding (Ids) and MafB. Overexpression of Ids or MafB in osteoclast precursors inhibits the expression of NFATc1 (Lee et al. 2006, Kim et al. 2007). In particular, Id proteins (Id1-Id4) negatively regulate the binding of basic helix-loop-helix (bHLH) transcription factors (Benezra et al. 1990, Massari and Murre 2000), such as Mitf, to the DNA. Id2 directly binds to Mitf and inhibits Mitf-mediated OSCAR expression (Lee et al. 2006). These results indicated that RANKL-mediated downregulation of Ids and MafB is important for osteoclast differentiation.

Many naturally occurring products suppress the formation of osteoclasts (Putnam et al. 2007). Along with other researchers, we have reported that natural products produced by plants exert inhibitory effects on osteoclast differentiation and bone resorption (Kwak et al. 2008, Kim et al. 2009). Recently, we found that rotenone, which is derived from *Derris elliptica*, exerts an inhibitory effect on osteoclast differentiation and bone resorption in vivo. Rotenone suppresses osteoclast differentiation and bone resorption in vitro and in vivo (Kwak et al. 2010). Thus, the main aim of our study is to discover the drug that inhibits bone loss by suppressing osteoclast differentiation and bone resorption using rotenone derivate. In this study, we found that 1′,2′-dihydrorotenone inhibited osteoclast differentiation of BMMs derived from mouse bone marrow cells (BMCs) and did not exert cytotoxic effects on BMMs.

Materials and Methods

Reagents

Human soluble RANKL (Cat. No. 310-01) and macrophage-colony stimulating factor (M-CSF; Cat. No. 300-25) were from PeproTech EC (London, UK). 1′,2′-dihydrorotenone was from Maybridge Chemical Co. (Tintagel, Cornwall, UK). ICR mice were from Dae Han Biolink Co. Ltd. (Chungbuk, Korea). The XTT assay kit was from Roche (Indianapolis, IN, USA; Cat. No. 11465015001). Lipopolysaccharide (LPS) (*Escherichia coli* 026:B6; Cat. No. L8274), 1α,25-dihydroxyvitamin D_3 (Vit D_3; Cat. No. D1530), prostaglandin E_2 (PGE_2; Cat. No. P5640), and anti-actin antibody (Cat. no. A5316) were from Sigma-Aldrich (St. Louis, MO, USA). Antibodies against c-Fos (Cat. no. SC253) and NFATc1 (Cat. no. SC7294) were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

Osteoclast differentiation

To isolate BMMs, we first isolated BMCs from 5-week-old male ICR mice by flushing the bone marrow cavity of long bones with α-minimum essential medium (α-MEM; Gibco/invitrogen, Carlsbad, CA, USA; Cat. no. 12571048) containing antibiotics (Sigma; Cat. no. P4333). BMCs were harvested by centrifugation at 1,600 rpm and culturing in α-MEM supplemented with 10% fetal bovine serum (FBS; Welgene, Daegu, Korea; Cat. no. S 001-03) and antibiotics for 1 day. Nonadherent cells were collected, seeded on 90-mm petri dishes, and cultured for 3 days in the presence of M-CSF (30 ng/mL). Subsequently, the nonadherent cells were removed and adherent cells were used as BMMs. The BMMs were further cultured for 4 days with M-CSF (30 ng/mL) and RANKL (50 ng/mL) in the presence of 1′,2′-dihydrorotenone.

Cytotoxicity assay

BMMs were seeded at a density of 1,000 cells/well in 96-well plates containing 200 μL of medium, and the cells were treated with 1′,2′-dihydrorotenone, as indicated in

the figure 1C. After culturing for 3 days, XTT reagent was added to the medium and the cells were incubated for 4 h. The optical density at 450 nm was determined using an enzyme-linked immunosorbent assay (ELISA) reader.

Western blotting

After various treatments as indicated in the figure, the cells were washed with ice-cold phosphate-buffer saline (PBS) and lysed in lysis buffer (50 mM Tris-Cl, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 1 mM sodium fluoride, 1 mM sodium vanadate, 1% deoxycholate, and 200 mM PMSF). The cell lysates were separated by centrifugation, and the supernatant was boiled in sodium dodecyl sulfate (SDS) sample buffer. The samples were subjected to 10% SDS-polyacrylamide gel electrophoresis (PAGE) and then transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% skim milk, washed, and then incubated with a primary antibody, as indicated. The membrane was incubated with peroxidase-conjugated anti-mouse or anti-rabbit secondary antibody, washed, and then visualized by using enhanced chemiluminescence.

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

The total RNA was isolated from cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). One microgram of RNA was reverse transcribed into cDNA using Superscript II reverse transcriptase, oligo dT primers, dNTP, buffer, dithiothreitol, and RNase inhibitor. cDNA was amplified by polymerase chain reaction (PCR). The sequences of the primers used for RT-PCR are listed in Table 1. PCR products were electrophoresed on 1% agarose gels and visualized by staining with ethidium bromide.

Bone resorption

To isolate mature osteoclasts, we isolated calvarial osteoblasts from the calvariae of newborn mice according to previously described methods (Kwak et al. 2008). BMCs and calvarial osteoblasts were co-cultured on a collagen-coated 90-mm dish in the presence of $VitD_3$ and PGE_2. After 6 days, the cultured cells were harvested by 0.1% collagenase digestion, seeded on hydroxyapatite-coated 48-well plates (Osteogenic Core Technologies, Cheonan, Korea), and then treated for 12 h with 1′,2′-dihydrorotenone. To measure the resorption activity, we removed the cells and photographed the plates under a light microscope. The resorption area was measured using the Pro-plus program, version 4.0 (Media Cybernetics)

LPS-induced bone erosion

LPS was used to induce in vivo bone erosion according to previously described methods (Kwak et al. 2010). Briefly, 1 day before the injection of LPS (5 μg/g of body weight) and subsequently on every alternate day for up to 8 days till the end of the experimental period, intraperitoneal injections of 1′,2′-dihydrorotenone (1 μg/g of body weight) were administered. In control mice, a suspension of 1% dimethylsulfoxide (DMSO) in PBS was injected in place of LPS. LPS was injected intraperitoneally on days 0 and 4. All mice were killed by cervical dislocation, and their femurs were imaged by a micro-computed tomography (CT) (NFR-Polaris-S160; Nano Focus Ray, Iksan, Korea). The bone volume to tissue volume ratio (BV/TV) was measured to assess the trabecular bone microstructure of the femur.

Statistical analysis

All quantitative data are presented as the mean (standard

Table 1. Primers used in this study

Gene	Forward (5′-3′)	Reverse (5′-3′)
TRAP	ACTTCCCCAGCCCTTACTAC	TCAGCACATAGCCCACACCG
c-Fos	CTGGTGCAGCCCACTCTGGTC	CTTTCAGCAGATTGGCAATCTC
NFATc1	CAACGCCCTGACCACCGATAG	GGCTGCCTTCCGTCTCATAGT
OSCAR	GAACACCAGAGGCTATGACTGTTC	CCGTGGAGCTGAGGAAAAGGTTG
Id1	GTCTGTCGGAGCAAAGCGTG	ACACAAGATGCGATCGTCGG
Id2	AAGGAACTGGTGCCCAGCAT	CAAGACACCTGGGCAAGACG
Id3	GGAGCTTTTGCCACTGACCC	CCCATTCTCGGAAAAGCCAG
MafB	AGCAGGTGTGACTCACGATG	CCTTGTAGGCGTCTCTCTCG
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA

deviation, S.D.) of 3-5 replicate experiments. The statistical differences were analyzed by Student's t-test. Statistically significant data (p values < 0.05) are indicated with an asterisk.

Results

1′,2′-dihydrorotenone suppresses the differentiation of BMMs into osteoclasts

To examine whether 1′,2′-dihydrorotenone, a chemical derivative of rotenone (Fig. 1A), regulates RANKL-induced osteoclast differentiation, we cultured BMMs derived from mouse BMCs for 4 days with RANKL and M-CSF in the presence of 1′,2′-dihydrorotenone. RANKL significantly induced TRAP-positive osteoclast differentiation. However, 1′,2′-dihydrorotenone considerably reduced the number of TRAP-positive osteoclasts in a dose-dependent manner and inhibited RANKL-induced osteoclast differentiation (Fig. 1B and 1C). To exclude the possibility that 1′,2′-dihydrorotenone inhibited osteoclast differentiation because it was toxic to BMMs, BMMs were incubated with 1′,2′-dihydrorotenone at the concentrations used in this study in the presence of M-CSF and cultured for 3 days. 1′,2′-

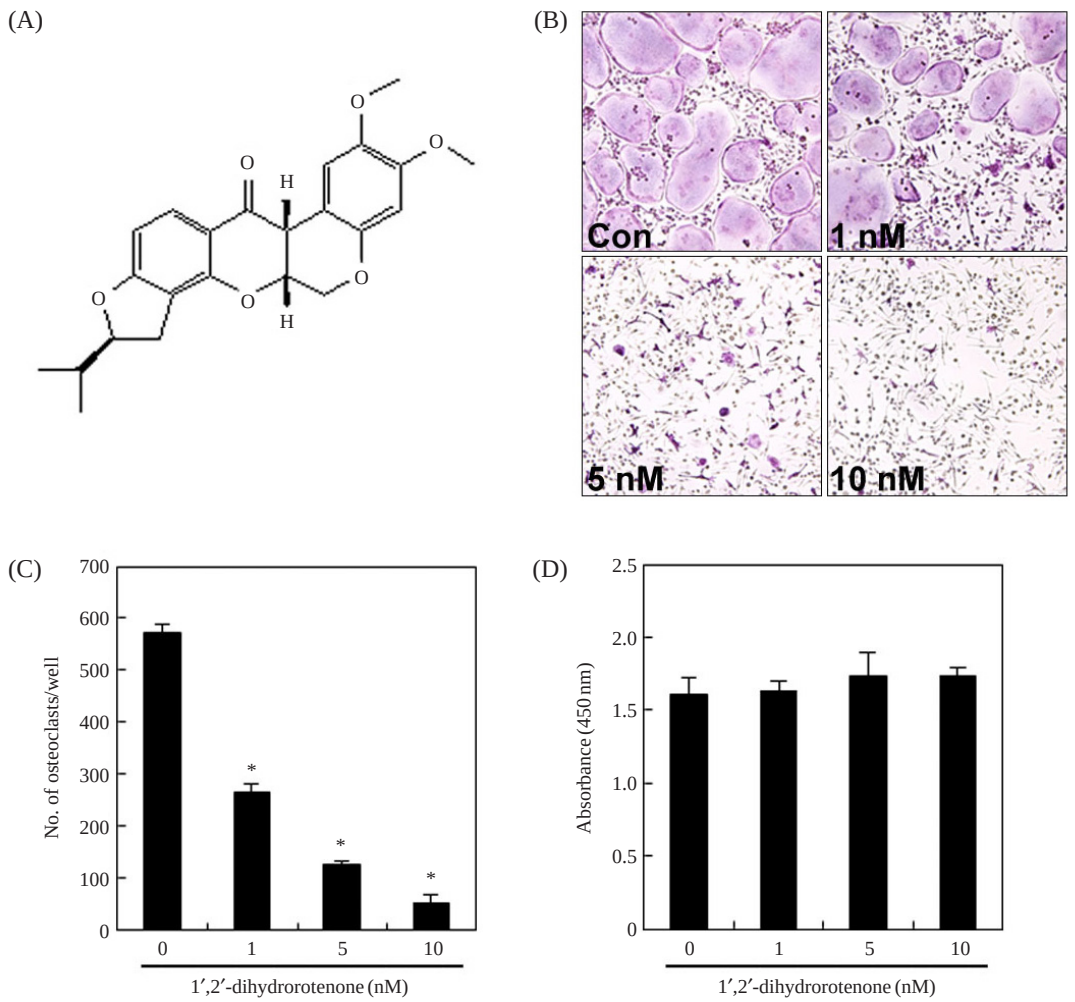

Fig. 1. Effect of 1′,2′-dihydrorotenone on osteoclast differentiation. (A) The chemical structure of 1′,2′-dihydrorotenone. (B) BMMs were cultured with RANKL (100 ng/mL) and M-CSF (30 ng/mL) in the presence of the indicated concentrations of 1′,2′-dihydrorotenone for 4 days. Cells were fixed in 3.7% formalin, permeabilized in 0.1% Triton X-100, and finally stained for TRAP. (C) TRAP-positive cells were counted as osteoclasts. 1′,2′-dihydrorotenone exerts a negative effect on osteoclast differentiation. Asterisks indicate the statistical difference ($p < 0.01$) from control (without 1′,2′-dihydrorotenone). (D) BMMs were seeded into a 96-well plate and cultured with M-CSF (30 ng/mL) in the presence of the indicated concentrations of 1′,2′-dihydrorotenone for 3 days. XTT reagent was added to each well, and the plates were incubated for 4 h. Absorbance was measured using an ELISA reader. 1′,2′-dihydrorotenone had no cytotoxic effects.

dihydrorotenone did not exert cytotoxic effects as compared to the solvent control (Fig. 1D). These results suggest that 1′,2′-dihydrorotenone inhibited RANKL-mediated osteoclast differentiation without reducing the viability of BMMs.

1′,2′-dihydrorotenone inhibits RANKL-induced c-Fos and NFATc1 expression

NFATc1 is a master regulator of terminal differentiation of osteoclasts (Takayanagi 2007). NFATc1 induces the expression of various osteoclast-specific genes such as TRAP and OSCAR. Thus, we examined whether 1′,2′-dihydrorotenone-mediated inhibition of osteoclast differentiation involves the suppression of RANKL-induced gene expression in BMMs treated with 1′,2′-dihydrorotenone. 1′,2′-dihydrorotenone inhibited the mRNA expression of c-Fos, NFATc1, TRAP, and OSCAR in BMMs treated with RANKL (Fig. 2A). To confirm this finding, we analyzed the protein levels of c-Fos and NFATc1 by western blotting. 1′,2′-dihydrorotenone suppressed the protein expression of c-Fos and NFATc1 in BMMs treated with RANKL (Fig. 2B). These results suggest that the inhibition of osteoclast differentiation by 1′,2′-dihydrorotenone may involve the inhibition of RANKL-induced gene expression. Next, we examined the expression levels of Ids and MafB, which are the negative regulators of osteoclast differentiation, during osteoclast differentiation. The expressions of Id1, 2, and 3, and MafB were significantly suppressed in BMMs treated with RANKL; however, the expressions of Id1 and 2 were sustained in BMMs treated with both RANKL and 1′,2′-dihydrorotenone (Fig. 2C). Our results suggest that 1′,2′-dihydrorotenone may influence the expression levels of c-Fos, NFATc1, and Ids during osteoclast differentiation.

1′,2′-dihydrorotenone suppresses osteoclastic bone resorption

To examine whether 1′,2′-dihydrorotenone inhibits osteoclast function with osteoclast differentiation, we performed bone resorption assays using mature osteoclasts. Mature osteoclasts were seeded on hydroxyapatite plates and then treated with 1′,2′-dihydrorotenone. As shown in Fig. 3, mature osteoclasts induced bone resorption, but the resorption pits and area were greatly inhibited after treatment with 1′,2′-dihydrorotenone when compared with those in the control. These results indicate that 1′,2′-dihydrorotenone

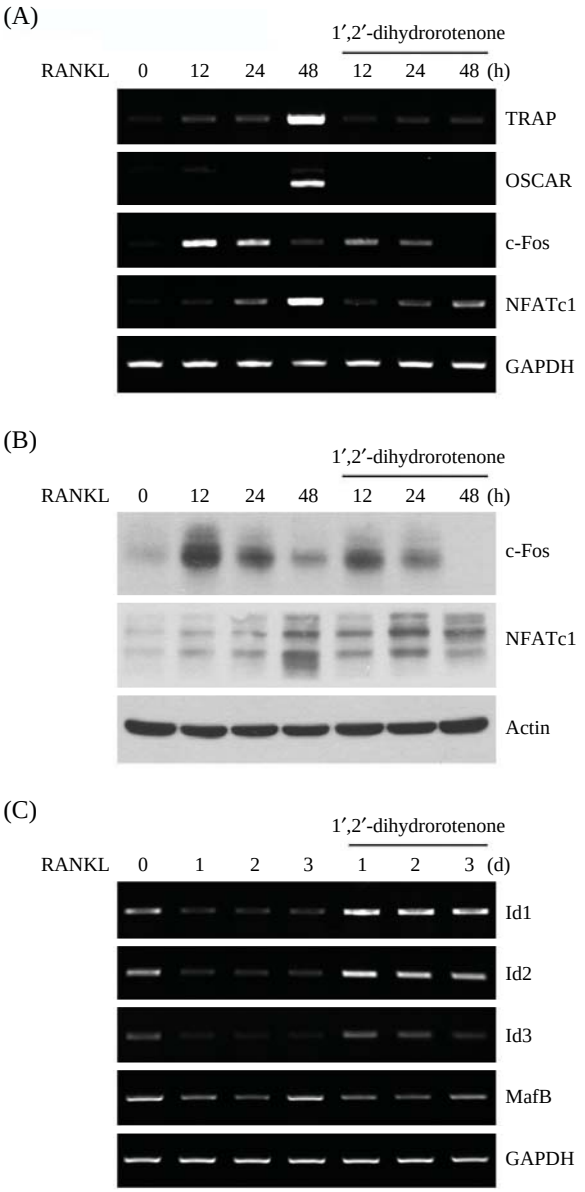

Fig. 2. 1′,2′-dihydrorotenone inhibits RANKL-mediated gene expression. (A) BMMs were pretreated with 1′,2′-dihydrorotenone (10 nM) for 1 h and then stimulated with RANKL (100 ng/mL) for the indicated time. RNA was isolated from treated cells, and the mRNA expression of the indicated genes was analyzed by RT-PCR. The mRNA expression of TRAP, OSCAR, c-Fos, and NFATc1 induced by RANKL was decreased in the presence of 1′,2′-dihydrorotenone. (B) BMMs were treated as in (A). Cell lysates were resolved by SDS-PAGE and western blotting was performed with anti-c-Fos, anti-NFATc1, and anti-actin antibodies as indicated. 1′,2′-dihydrorotenone inhibited the expression of c-Fos and NFATc1 induced by RANKL. (C) BMMs were pretreated with 1′,2′-dihydrorotenone (10 nM) for 1 h and then stimulated with RANKL (100 ng/mL) for the indicated time. The mRNA expression of the indicated genes was analyzed by RT-PCR. The levels of Id1 and Id2 mRNA regulated by RANKL were decreased by 1′,2′-dihydrorotenone treatment.

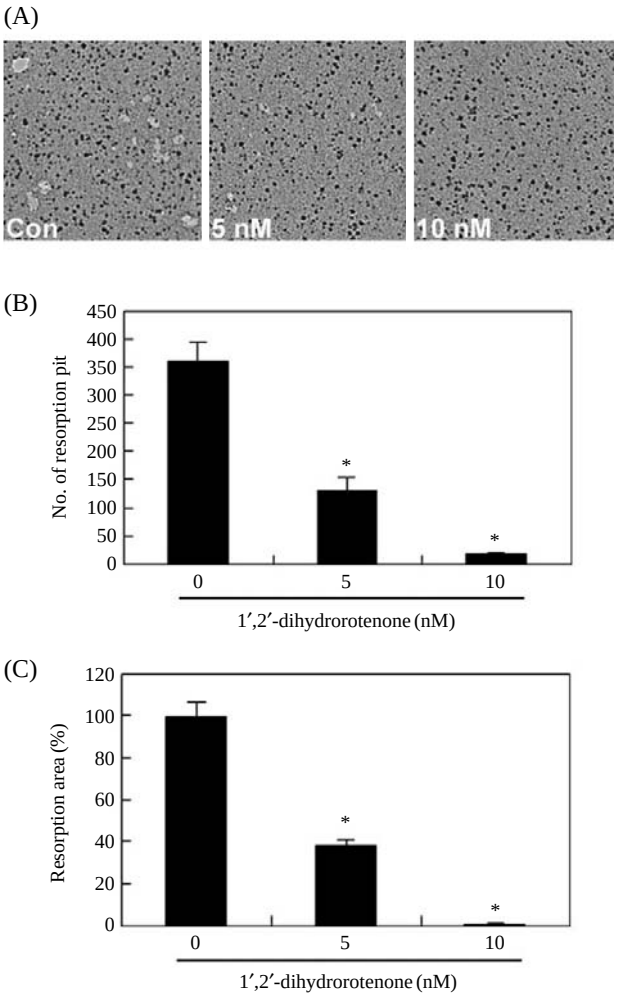

Fig. 3. 1′,2′-dihydrorotenone can inhibit bone resorption. Mature osteoclasts were obtained by coculturing bone marrow cells and calvarial osteoblasts. (A) Mature osteoclasts were seeded on hydroxyapatite-coated 48-well plates and further stimulated for 12 h with the indicated concentrations of 1′,2′-dihydrorotenone. The cells were removed from the plates and photographed under a light microscope. The pit number (B) and pit area (C) were determined using Image Pro-plus program, version 4.0. Asterisks indicate the statistical difference ($p < 0.01$) from control (without 1′,2′-dihydrorotenone). The bone resorption pits and area were decreased by 1′,2′-dihydrorotenone.

can suppress bone resorption as well as osteoclast differentiation.

1′,2′-dihydrorotenone suppresses inflammatory bone loss

Next, we examined the in vivo effect of 1′,2′-dihydrorotenone in a mouse model of bone-related diseases. To induce bone erosion in the mouse model, we intraperitoneally injected the mice with LPS and 1′,2′-dihydrorotenone on alternate days. Micro-CT analysis showed that LPS induced bone erosion, but this effect was inhibited by treatment with 1′,2′-dihydrorotenone (Fig. 4A). The BV/TV index suggested that the trabecular bone mass in the mice injected with both LPS and 1′,2′-dihydrorotenone was less than that in mice injected with LPS only (Fig. 4B). These results indicate that 1′,2′-dihydrorotenone suppressed osteoclastic bone erosion in vivo.

Discussion

Osteoclasts differentiate through cell-to-cell interactions from monocyte/macrophage lineages and osteoblastic cells (Boyle et al. 2003). BMMs differentiate into osteoclasts in response to treatment with both RANKL and M-CSF in vitro. In this study, we examined the effect of 1′,2′-dihydrorotenone on the differentiation of BMMs to osteoclasts. BMMs differentiate into TRAP-positive osteoclasts in the presence of M-CSF and RANKL; however, M-CSF- and RANKL-induced osteoclast formation was significantly inhibited in the presence of 1′,2′-dihydrorotenone (Fig. 1). 1′,2′-dihydrorotenone exerts a negative effect on osteoclast differentiation. To exclude the possibility that 1′,2′-dihydrorotenone inhibited osteoclast differentiation because it was cytotoxic to BMMs, we incubated BMMs with 1′,2′-dihydrorotenone at the concentrations used in this study in the presence of M-CSF and cultured for 3 days. 1′,2′-dihydrorotenone-mediated inhibition of osteoclast differentiation was not due to the toxic effects of 1′,2′-dihydrorotenone (Fig. 1). To understand the molecular mechanism by which 1′,2′-dihydrorotenone suppressed osteoclast differentiation, we first analyzed the effect of 1′,2′-dihydrorotenone on the expression of osteoclast-specific genes in BMMs treated with RANKL. In the presence of 1′,2′-dihydrorotenone, the expression levels of c-Fos, NFATc1, TRAP, and OSCAR were decreased in BMMs treated with RANKL (Fig. 2). These results suggest that 1′,2′-dihydrorotenone suppresses osteoclast differentiation by inhibiting c-Fos and NFATc1 expression.

RANKL stimulates transcription factors such as NF-κB, MITF, and AP-1 (Takayanagi 2007). In particular, RANK signaling pathway induces the expression of the immediate-early gene c-Fos (Matsuo et al. 2000). Regulation of c-Fos expression is important for determining the fate of

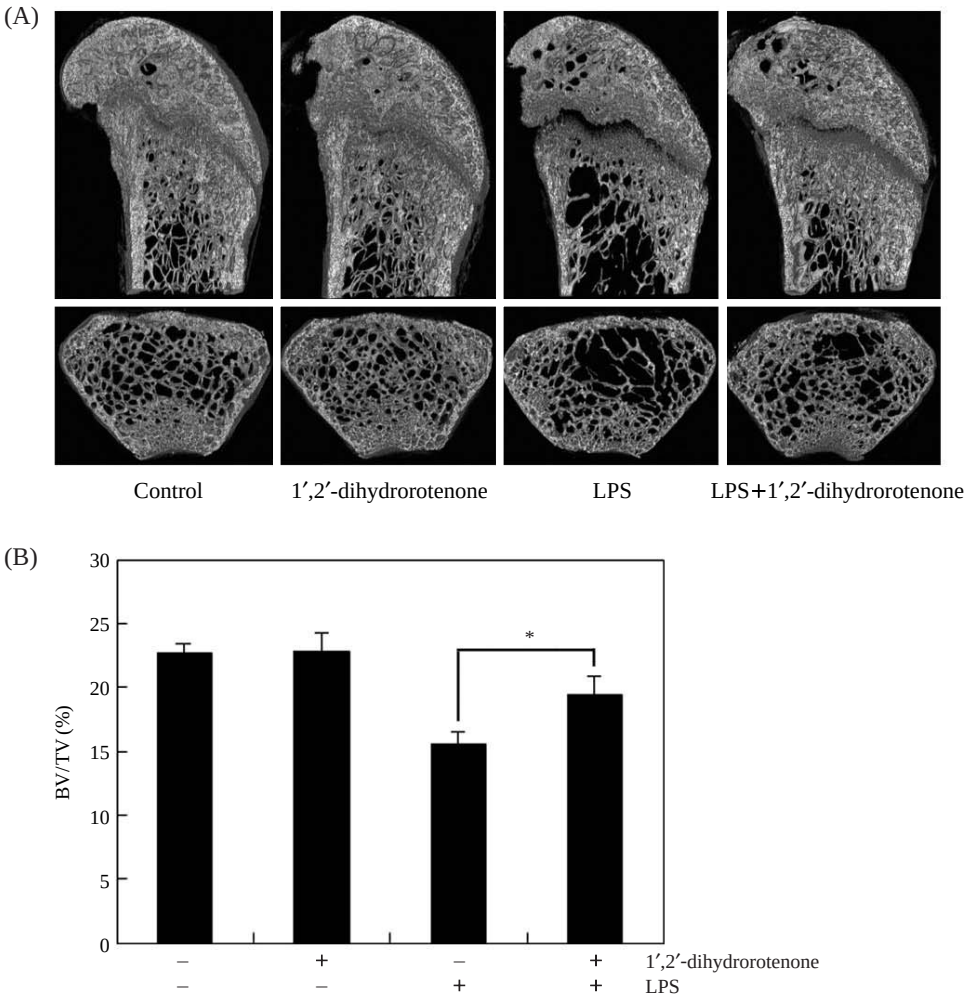

Fig. 4. 1′,2′-dihydrorotenone inhibits osteoclastic bone resorption in vivo. 1′,2′-dihydrorotenone (1 μg/g of body weight) was injected intraperitoneally into mice 1 day before the injection of LPS, and for the next 8 days, intraperitoneal injections of 1′,2′-dihydrorotenone were given on alternate days. LPS or PBS (control) were injected intraperitoneally on days 0 and 4 in controls. Femurs were obtained on day 8 after the first injection of LPS, and radiographs were obtained with a micro-CT apparatus. LPS-induced bone erosion was suppressed by treatment with 1′,2′-dihydrorotenone. (B) Bone volume/tissue volume (BV/TV; %) was determined from the micro-CT data by using the software provided by VGStudio MAX 1.2 software. Asterisks indicate a statistically significant difference between groups ($p < 0.05$). Alteration of bone mass caused by LPS was reversed by treatment with 1′,2′-dihydrorotenone.

BMMs. For example, the induction of c-Fos contributes to osteoclast differentiation but inhibits macrophage differentiation. Although the mechanism by RANKL acts to induce c-Fos expression during osteoclast differentiation remains unclear, it has been reported that p50 and p65 NF-κB subunits play important roles in c-Fos expression (Yamashita et al. 2007). Otero et al (Otero et al. 2010) recently reported that BMMs transduced with constitutively active (CA)-IKK-β exhibited RANKL-independent expression of c-Fos and differentiated into osteoclasts in the absence of RANKL. These results indicated that the NF-κB pathway is an important upstream pathway that induces c-Fos expression in BMMs. Thus, to determine the mechanism of 1′,2′-dihydrorotenone-mediated c-Fos inhibition, we examined the effect of 1′,2′-dihydrorotenone on RANKL-induced I-κB degradation as well as on mitogen-activated protein kinase (MAPK) activation. 1′,2′-dihydrorotenone did not affect RANKL-mediated degradation of I-κB and MAPK activation (data not shown). Thus, the mechanism of 1′,2′-dihydrorotenone-mediated c-Fos inhibition remains to be determined. Recently, it was reported that RANKL induces osteoclast differentiation by inhibiting Ids expres-

sion (Lee et al. 2006). Therefore, we investigated whether the inhibitory effect of 1′,2′-dihydrorotenone on osteoclast differentiation influenced the level of Ids. Although Ids did not regulate RANKL-induced c-Fos expression, the expression of NFATc1 was restricted to osteoclast precursors transduced with Id2 (Lee et al. 2006). We found that RANKL-mediated inhibition of Id1 and 2 was reversed by 1′,2′-dihydrorotenone treatment (Fig. 2). Taken together, our results suggest that 1′,2′-dihydrorotenone-mediated expression of c-Fos and Ids may influence the inhibition of osteoclast differentiation.

Osteoclastic bone resorption is the primary mechanism responsible for bone diseases such as osteoporosis and rheumatoid arthritis (Walsh et al. 2006). Inhibition of bone resorption is important for treating the ongoing bone erosion in patients with bone-related diseases. Thus, to examine the effect of 1′,2′-dihydrorotenone on bone resorption, we assessed bone resorption by mature osteoclasts derived from co-cultures of bone marrow cells and calvarial osteoblasts. 1′,2′-dihydrorotenone considerably inhibited bone resorption by mature osteoclasts (Fig. 3). LPS, a potent inducer of inflammation, induced in vivo bone loss, although the mechanism of this effect remains unknown (Miyaura et al. 2003). Various inflammatory factors can induce osteoclast formation and bone resorption, both directly and indirectly (Kobayashi et al. 2000, Takayanagi et al. 2000, Tanabe et al. 2005). Importantly, LPS-injected mice showed a profound decrease in the density of trabeculae in the femur, and 1′,2′-dihydrorotenone-injected mice showed a marked reduction in bone erosion (Fig. 4). Our results suggest that 1′,2′-dihydrorotenone inhibits osteoclastic bone resorption as well as differentiation into osteoclasts in vivo.

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세포와 생체 실험에서 파골세포 분화와 골 흡수에 대한 1′,2′-dihydrorotenone의 저해 효과

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간추림 : 뼈 관련 질환을 앓고 있는 환자에게 부작용이 없는 치료제의 발견은 중요하다. 따라서 본 연구의 목적은 뼈 파괴세포의 분화와 뼈 흡수를 억제할 수 있는 새로운 물질을 발견하는 것이다. 본 연구에서 뼈 파괴세포의 분화 및 뼈 흡수에 1′,2′-dihydrorotenone의 효과를 세포수준과 마우스 생체 내에서 검증하였다. 1′,2′-dihydrorotenone은 농도 증감에 따라 receptor activator of NF-κB ligand (RANKL)에 의해 대식세포에서 뼈 파괴세포로의 분화를 억제하였다. 그러나 1′,2′-dihydrorotenone은 대식세포에 독성을 나타내지 않았다. 이들의 결과로 1′,2′-dihydrorotenone이 대식세포로부터 뼈 파괴세포로 분화에 특이적으로 작용한다고 할 수 있다. 1′,2′-dihydrorotenone은 RANKL에 의한 뼈 파괴세포 특이 유전자 뿐만 아니라 c-Fos와 NFATc1의 발현을 억제하였다. RANKL는 inhibitors of differentiation/DNA binding (Id)1, 2, 3의 발현을 억제하였지만, 1′,2′-dihydrorotenone가 Id1과 Id2의 발현을 촉진하였다. 더욱이, 1′,2′-dihydrorotenone은 뼈 파괴세포의 뼈 흡수를 억제하였고 마우스 생체 내 lipopolysaccharide에 의한 뼈 흡수를 억제하였다. 이 모든 결과로 1′,2′-dihydrorotenone이 뼈 질환 치료제로 가능성을 가진다고 할 수 있다.

찾아보기 낱말 : 뼈 파괴세포, RANKL, 1′,2′-dihydrorotenone, 뼈 흡수

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