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Olive polyphenol hydroxytyrosol prevents bone loss

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ABSTRACT

Polyphenols reportedly exert physiological effects against diseases such as cancer, arteriosclerosis, hyperlipidemia and osteoporosis. The present study was designed to evaluate the effects of oleuropein, hydroxytyrosol and tyrosol, the major polyphenols in olives, on bone formation using cultured osteoblasts and osteoclasts, and on bone loss in ovariectomized mice. No polyphenols markedly affected the proliferation of osteoblastic MC3T3-E1 cells at concentrations up to 10 μM. Oleuropein and hydroxytyrosol at 10 to 100 μM had no effect on the production of type I collagen and the activity of alkaline phosphatase in MC3T3-E1 cells, but stimulated the deposition of calcium in a dose-dependent manner. In contrast, oleuropein at 10 to 100 μM and hydroxytyrosol at 50 to 100 μM inhibited the formation of multinucleated osteoclasts in a dose-dependent manner. Furthermore, both compounds suppressed the bone loss of trabecular bone in femurs of ovariectomized mice (6-week-old BALB/c female mice), while hydroxytyrosol attenuated H_2O_2 levels in MC3T3-E1 cells. Our findings indicate that the olive polyphenols oleuropein and hydroxytyrosol may have critical effects on the formation and maintenance of bone, and can be used as effective remedies in the treatment of osteoporosis symptoms.

1. Introduction

In bone tissues, both the formation and maintenance of bone are controlled by bone-forming osteoblasts and bone-resorbing osteoclasts, and an imbalance between these two types of cell leads to the bone metabolic diseases, such as osteoporosis and osteopetrosis (Riggs, 1987). The formation of bone involves a complex series of events that include the proliferation and differentiation of osteoprogenitor cells and eventually result in the formation of a mineralized extracellular matrix. The sequential expression of type I collagen, alkaline phosphatase (ALPase) and osteocalcin, and the deposition of calcium are known as markers of osteoblastic differentiation. Several model systems have been developed for studies of the proliferation and differentiation of bone-forming cells in vitro and the molecular biology of the mineralization process, such as preosteoblastic cells from mouse calvariae (MC3T3-E1 cells) and osteoblast-like cells from rat calvariae (Bredford et al., 1993; Hagiwara et al., 1996; Liu et al., 1994; Stein et al., 1990). On the other hand, osteoclasts are multinucleated giant cells with the ability to resorb mineralized tissues. They are formed from hematopoietic cells of the monocyte/macrophage lineage (Udagawa et al., 1990). The development of osteoclasts in culture is strictly dependent on support provided by osteoblasts and/or stromal cells (Udagawa et al., 1990). The formation and activation of osteoclasts are controlled by the combined action of the receptor activator of nuclear factor-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). Here, we used a single culture of mouse spleen cells with the addition of M-CSF and soluble RANKL (Notoya et al., 2007).

In in vivo experiments, we utilized ovariectomized (OVX) mice as an osteoporosis model. As an animal model for postmenopausal bone loss, OVX mice or rats have long been used (Kalu, 1991; Puel et al., 2006). The decline in sex hormones at the time of menopause is known to heighten the risk of osteoporosis (Ershler et al., 1997).

Polyphenols display antioxidant and anti-inflammatory properties. Recent reports have suggested that reactive oxygen species play an important role in the regulation of cell proliferation, differentiation and metabolism. In particular, reactive oxygen species inhibit the formation of bone by osteoblastic cells (Hosoya et al., 1998; Lee et al., 2006; Mody et al., 2001). We previously reported that quercetin (Notoya et al., 2004) and curcumin (Notoya et al., 2006) inhibit the mineralization of osteoblastic cells and the formation of osteoclasts. Furthermore, curcumin attenuates bone loss in the femurs of OVX mice (Notoya et al., 2006). The present study was designed to evaluate the in vitro and in vivo effects of oleuropein and hydroxytyrosol on the formation and maintenance of bone using cultured cells and ovariectomized mice. Our

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results indicate that olive polyphenols may be useful in the prevention and treatment of osteoporosis.

2. Materials and methods

2.1. Materials

Oleuropein, hydroxytyrosol and tyrosol were purchased from Funakoshi (Tokyo, Japan). 1α, 25-dihydroxy-vitamin D_3 [1α, 25$(OH)_2$ vitamin D_3] was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). α-Modified minimum essential medium (α-MEM), RPMI 1640 medium, and penicillin/streptomycin antibiotic mixture were obtained from Life Technologies, Inc. (Grand Island, NY, USA). Fetal bovine serum was obtained from Moregate BioTech (Bulimba, Australia). Recombinant murine M-CSF and recombinant human soluble RANKL (sRANKL) were from R&D Systems (Minneapolis, MN, USA) and Pepro Tech EC., Ltd. (London, UK), respectively.

2.2. Culture of osteoblastic cells

Preosteoblastic MC3T3-E1 cells were obtained from RIKEN Cell Bank (Tsukuba, Japan). Cells were maintained in a 55-cm^2 dish in α-MEM, supplemented with 10% fetal bovine serum, 50 units/ml penicillin and 50 μg/ml streptomycin, in a humidified atmosphere of 5% CO_2 in air at 37 °C. After reaching 70% confluence, cells were detached by treatment with 0.05% trypsin, replated in either 55-cm^2 dishes or 12-well plates (area of each well, 3.8 cm^2) at a density of 1×10^4 cells/cm^2 and grown in α-MEM supplemented with 10% fetal bovine serum, 50 units/ml penicillin, 50 μg/ml streptomycin, 5 mM β-glycerophosphate, and 50 μg/ml ascorbic acid. Fresh medium and olive polyphenols were supplied to cells at 2-day intervals. MC3T3-E1 cells formed nodules, and mineralization of nodules was observed after cultivation for 2 to 3 weeks.

2.3. Formation of osteoclastic cells

Spleen cells were collected from splenic tissues of 6-week-old male ddY mice (Sankyo Laboservice, Tokyo, Japan). Erythrocytes contaminating the spleen cell fraction were eliminated by adding 0.83% ammonium chloride in 10 mM Tris–HCl (pH 7.4) to the cell pellet. Mouse spleen cells (2.4×10^5 cells/well) in 96-well plates (0.32 cm^2/well) were cultured with 50 ng/ml human sRANKL and 30 ng/ml M-CSF for 7 days. Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air. Fresh medium and compounds were supplied at 2-day intervals. The Institutional Animal Care and Use Committee of Toin University of Yokohama approved the animal protocols and procedures.

Multinucleated osteoclastic cells were fixed in 3.7% formaldehyde for 5 min and then in a mixture of ethanol and acetone (1:1, v/v) for 1 min. These were then stained for tartrate-resistant acid phosphatase (TRAP) activity. TRAP-positive multinucleated cells (three or more nuclei) were counted under a microscope (IX70; Olympus, Tokyo, Japan).

2.4. Cell viability

MC3T3-E1 cells were replated in 96-well plates (area of each well, 0.32 cm^2) at the low density of 1×10^3 cells/cm^2 and grown in α-MEM supplemented with 10% fetal bovine serum, 50 units/ml penicillin, 50 μg/ml streptomycin, and olive polyphenol at various concentrations. After subculture for 2 to 3 days, cell layers were washed twice with RPMI 1640 medium. MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide; DOJINDO, Kumamoto, Japan) reagent (0.5 mg/ml RPMI 1640) was added to each well, followed by incubation for 4 h to form formazan. After removing the medium, dimethyl sulfoxide was added to each well to dissolve formazan, and formazan solution was measured at 570 nm.

2.5. Sirius Red staining of type I collagen

Sirius Red F3BA was purchased from Polysciences, Inc. (Wamington, PA, USA). Dye was dissolved in saturated aqueous picric acid at a concentration of 100 mg/100 ml. Cells were plated in 24-well plates at a density of 1×10^4 cells/cm^2 and subcultured with olive polyphenols for 3 days. Cell layers were air-dried overnight in a sterile bench and fixed with 1 ml of Bouin's solution (Sigma, St. Louis, MO, USA) for 1 h. Fixation fluid was removed by suction and culture plates were washed by immersion in running tap water for 15 min. Culture plates were air-dried and before adding 1 ml of Sirius Red dye reagent. Cells were stained for 1 h with shaking on a plate shaker. Stained cell layers were washed with 0.01 N hydrochloric acid to remove all non-bound dye, and stained material was dissolved in 0.5 ml of 0.1 N sodium hydroxide using a plate shaker for 30 min at room temperature, after which the dye solution was measured at 550 nm.

2.6. Measurement of alkaline phosphatase activity

MC3T3-E1 cells were subcultured in 12-well plates (3.8 cm^2/well) in α-MEM containing 10% fetal bovine serum, 5 mM β-glycerophosphate, and 50 μg/ml ascorbic acid. After cells had reached confluence (day 3), olive polyphenols were added to cultures at various concentrations for 12 days. Cells were washed with 10 mM Tris–HCl, pH 7.2, and were sonicated in 1 ml of 50 mM Tris–HCl (pH 7.2) containing 0.1% Triton X-100 and 2 mM $MgCl_2$ for 15 s with a sonicator (Ultrasonic Disruptor UD-201; Tomy Co., Tokyo, Japan). Alkaline phosphatase activity was determined using an established technique with *p*-nitrophenyl phosphate as the substrate. Protein concentrations were determined using BCA protein assay reagent (Pierce Chemical Co., Rockford, IL, USA) with bovine serum albumin as a standard.

2.7. Quantitation of calcium deposition

MC3T3-E1 cells were subcultured in α-MEM containing 10% fetal bovine serum, 5 mM β-glycerophosphate, and 50 μg/ml ascorbic acid. After cells had reached confluence (day 3), olive polyphenols were added at various concentrations to the culture medium and cells were subcultured for 15 days. The amount of calcium, deposited as hydroxyapatite in the cell layer, was measured as follows. Layers of cells in 12-well plates (3.8 cm^2/well) were washed with phosphate-buffered saline (pH 7.4, PBS; 20 mM sodium phosphate and 130 mM NaCl) and incubated overnight with 1 ml of 2 N HCl with gentle shaking. The Ca^{2+} ions in the samples were quantitated by the *o*-cresolphthalein complexone method with a Calcium C kit (Wako Pure Chemical Industries). This kit is specific for Ca^{2+} ions and has a detection limit of 1 μg/ml. We used the solution of Ca^{2+} ions (20 mg/dl) in the kit as the standard solution.

2.8. Measurement of intracellular reactive oxygen species

Intracellular reactive oxygen species were measured using the oxidant-sensitive probe 2′, 7′-dichlorofluorescin diacetate (DCFH-DA). Cells (4×10^4 cells/dish) in 3.5-cm dishes were cultured for 48 h followed by a 48 h-treatment with olive polyphenol. Thereafter, cells were incubated with 50 μM of DCF-DA for 30 min with subsequent incubation with 500 μM of H_2O_2 for 30 min and washed with Hank's Balanced Salt Solution. Fluorescence emission was detected by confocal laser scanning microscopy at excitation and emission wavelengths of 488 nm and 505 nm, respectively. Images were analyzed using a confocal scanning system (TCP SP2; Leica, Tokyo, Japan).

2.9. Analysis of bone density of OVX mice treated with olive polyphenols

BALB/c female mice (6 weeks old) were purchased from Japan SLC Co. (Hamamatsu, Japan) and were housed individually at 24 °C with a

12 h light–dark cycle. Mice underwent sham-operation (n = 7) or were surgically ovariectomized (OVX; n = 28) under anesthesia by Nembutal® Injection (Dainippon Sumitomo Pharma, Tokyo, Japan). Mice were assigned to five groups (each n = 7): (1) untreated (Sham: sham-operated controls); (2) untreated (OVX controls); (3) OVX administered orally with oleuropein (10 mg/kg body weight) at 3-day intervals; (4) OVX administered orally with hydroxytyrosol (10 mg/kg body weight) at 3-day intervals; (5) OVX administered orally with tyrosol (10 mg/kg body weight) at 3-day intervals. Olive polyphenols were dissolved in corn oil, and corn oil alone was administered to untreated mice. The volume ingested orally was 100 μl. After the 28-day experimental period, left and right femurs were surgically collected from the anesthetized mice. The success of ovariectomy was confirmed by uterine atrophy in OVX mice.

Bone mineral density of femurs was assessed with X-ray CT System (LA Theata LCT-100; Aloka, Tokyo, Japan). We monitored the bone mineral density of femurs at 0.3 mm intervals and separately analyzed each trabecular and cortical bone. Values at 1.8 mm from the epiphysis of femur are shown in the figures. Animal protocols and procedures were approved by the Institutional Animal Care and Use Committee of Toin University of Yokohama.

2.10. Statistical analysis

Numerical data are expressed as means ± S.D. of the results from three or four cultures, and the significance of differences was analyzed by ANOVA Dunnett's test. Statistical significance was set at $P<0.05$. Experiments were repeated independently in triplicate and the results were qualitatively identical in every case. Results from representative experiments are shown.

3. Results

3.1. Effects of polyphenol on cultured osteoblasts and osteoclasts

Fig. 1 shows the structures of oleuropein and its metabolites. The products of oleuropein hydrolysis are the polar compounds hydroxytyrosol and tyrosol (Vissers et al., 2002).

We evaluated the viability of MC3T3-E1 cells treated with olive polyphenols using MTT assay. Exposure of MC3T3-E1 cells to 100 μM oleuropein and hydroxytyrosol decreased cell viability by approximately 45% and 70%, respectively, at 69 h, relative to control cultures treated with vehicle alone (Fig. 2). The effects of oleuropein and hydroxytyrosol were dose-dependent. Exposure of MC3T3-E1 cells to both compounds at 100 μM did not affect cell morphology. Even at 100 μM, tyrosol did not affect cell viability at 69 h (Fig. 2).

In order to assess the effects of olive polyphenol on differentiation and mineralization of MC3T3-E1 cells, we added each polyphenol to the culture medium of post-proliferative cells and assayed production of type I collagen, alkaline phosphatase activity, and calcium deposition (Fig. 3). Type I collagen is an early osteoblastic marker, and as shown in Fig. 3A, incubation of cells with each polyphenol did not affect production of type I collagen. We then examined the activity of alkaline phosphatase, a middle marker of osteoblastic differentiation, in MC3T3-E1 cells. None of the compounds affected the activity of alkaline phosphatase in MC3T3-E1 cells on day 15 (Fig. 3B). In

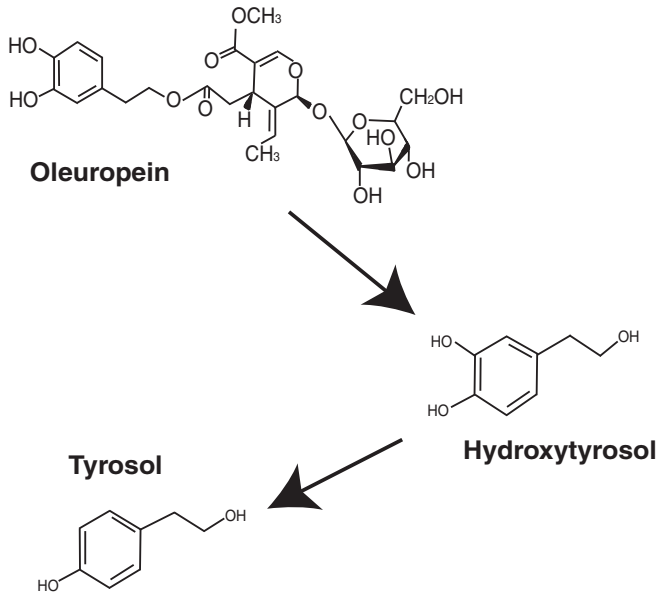

Fig. 1. Structure of oleuropein and its metabolites.

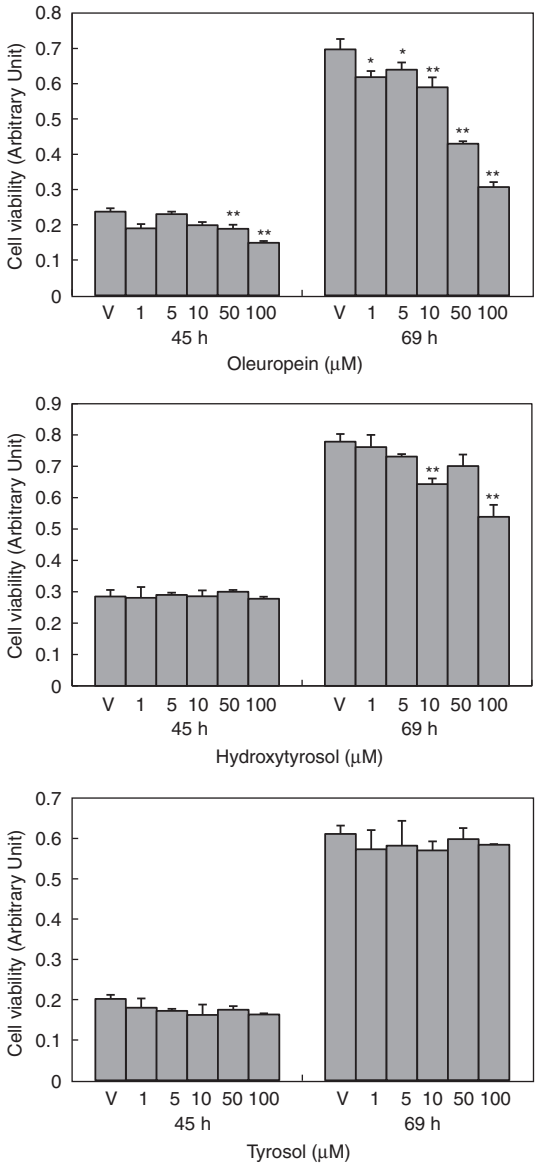

Fig. 2. Effects of olive polyphenol on viability of osteoblastic cells. MC3T3-E1 cells (1×10^3 cells/well; 96-well plates) were exposed to olive polyphenol at various concentrations (1 to 100 μM) and were subcultured for 45 or 69 h. After treatment with each compound, cells were treated with MTT (50 μg/well) for 4 h and absorbance at 570 nm was measured. Values represent means ± S.D. of results from three wells. Data are representative of results from three separate experiments. *, $P<0.05$ vs. vehicle (V).

contrast, as demonstrated in Fig. 3C, oleuropein and hydroxytyrosol enhanced the deposition of calcium ions by MC3T3-E1 cells on day 18.

We formed osteoclastic cells from mouse spleen cells by addition of 30 ng/ml M-CSF and 50 ng/ml human recombinant soluble RANKL. Fig. 4A shows representative results of staining for detection of TRAP activity in osteoclastic cells treated with each polyphenol at the indicated concentrations. Formation of TRAP-positive multinucleated osteoclastic cells was dose-dependently inhibited by the addition of oleuropein, hydroxytyrosol and tyrosol (Fig. 4B). Oleuropein was most effective for inhibition of osteoclast formation in culture, with 10 μM oleuropein inhibiting the formation of osteoclasts by 30%.

We examined the effects of olive polyphenol on hydrogen peroxide (H_2O_2) levels in MC3T3-E1 cells. As shown in Fig. 5, 50 μM hydroxytyrosol decreased in H_2O_2 levels by 60% in MC3T3-E1 cells. In contrast, oleuropein, tyrosol and apigenin at 50 μM did not affect H_2O_2 levels in the cells.

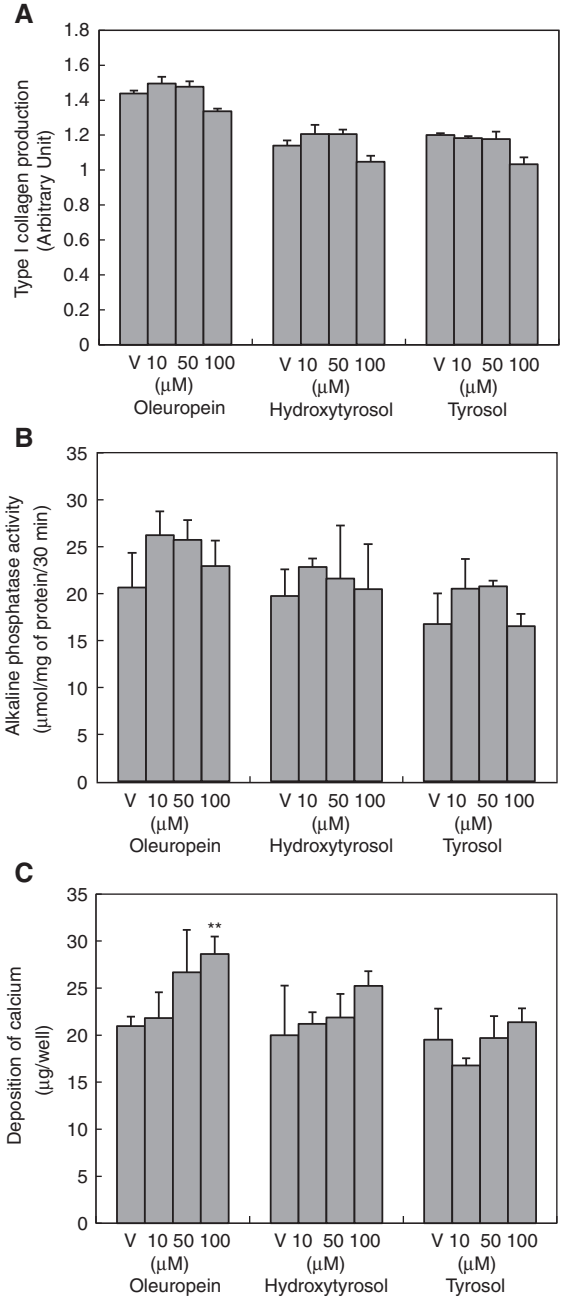

Fig. 3. Effects of olive polyphenol on the production of type I collagen, ALPase activity, and mineralization of osteoblasts. MC3T3-E1 cells were cultured in 12-well plates (3.8 cm^2/well) with α-MEM that contained 10% fetal bovine serum, 5 mM β-glycerophosphate, 50 μg/ml ascorbic acid. After cells reached confluence (day 3), olive polyphenol was added at various concentrations (1 to 100 μM) to culture medium. Fresh medium with test compound was supplied at three-day intervals. A: Type I collagen produced was measured at day 6 as described in the text. B: Alkaline phosphatase activity was measured at day 15 as described in the text. C: Deposition of Ca^{2+} ions was measured at day 18. Quantitative analysis of Ca^{2+} ions was performed as described in the text. All values represent means ± S.D. of results from three wells. Data are representative of results from three separate experiments. *, $P<0.05$ vs. vehicle (V) and **, $P<0.01$ vs. vehicle (V).

3.2. Effects of polyphenols on bone loss in OVX mice

We examined the effects of olive polyphenols on bone mineral density using OVX mice. We decided to use the 10 mg/kg/body weight dose in polyphenols, because hydroxytyrosol of 30 and 100 mg/kg/body weight doses showed the decrease in bone mineral density of femur compared to OVX mice (data not shown). There were no differences in body weight among OVX mice administered polyphenols, OVX mice, and sham-mice. Ovariectomy induced a severe decrease in bone mineral density of trabecular bone (Fig. 6). Oral administration of oleuropein and hydroxytyrosol at 10 mg/kg significantly reduced the loss of trabecular bone in OVX mice (Fig. 6). Hydroxytyrosol was most effective among the olive polyphenols tested. In contrast, oleuropein and tyrosol did not significantly affect the loss of bone mineral density in cortical bone of OVX mice.

4. Discussion

In this study, we attempted to clarify the potential effects of the olive polyphenols oleuropein, hydroxytyrosol and tyrosol on bone metabolism, and we found that all compounds inhibited the formation of multinucleated osteoclasts in culture, and that oleuropein enhanced the deposition of calcium by osteoblasts. Furthermore, oleuropein and hydroxytyrosol decreased bone loss of femurs in OVX mice.

We screened natural and synthetic polyphenols for their ability to regulate the proliferation, differentiation and function of cultured osteoblasts and osteoclasts in order to identify factors that might cause, prevent or treat bone metabolic diseases such as osteoporosis and osteopetrosis. To date, we have reported that the isoflavone genistein attenuates osteoclastogenesis by decreasing levels of receptor activator NF-κB ligand mRNA in osteogenic/stromal cells (Yamagishi et al., 2001). Recently, quercetin (Notoya et al., 2004) and curcumin (Notoya et al., 2006) were reported to inhibit cultured osteoblast metabolism. We also showed that alkylphenols, such as nonylphenol and octylphenol, inhibit the formation of osteoclasts in culture, and stimulate sternebrae bone calcification in fetal mice (Hagiwara et al., 2008). On the other hand, Kamon et al. (2010) reported that green tea polyphenol epigallocatechin gallate inhibited the differentiation of murine osteoblastic MC3T3-E1 cells and the formation of osteoclasts. Bharti et al. (2004) also reported that curcumin inhibits osteoclastogenesis. Thus, polyphenols regulate bone metabolism by acting on osteoblasts and osteoclasts in culture.

Olive leaves contain polyphenols, such as oleuropein, hydroxytyrosol, tyrosol and apigenin. Among these, hydroxytyrosol has strongest antioxidant effects. In the present study, we demonstrated that hydroxytyrosol effectively decreased H_2O_2 levels in MC3T3-E1 osteoblastic cells (Fig. 5). Oxidative stress resulting in increased levels of intracellular reactive oxygen species has been reported to suppress bone metabolism. Arai et al. (2007) reported that the mineralization levels by MC3T3-E1 cells were reduced by half after a single exposure to H_2O_2 within the non-toxic concentration range. In addition, there have been some reports that H_2O_2 suppresses differentiation markers, such as alkaline phosphatase activity and type I collagen gene expression, and mineralization of osteoblastic cells (Hosoya et al., 1998; Lee et al., 2006; Mody et al., 2001). On the

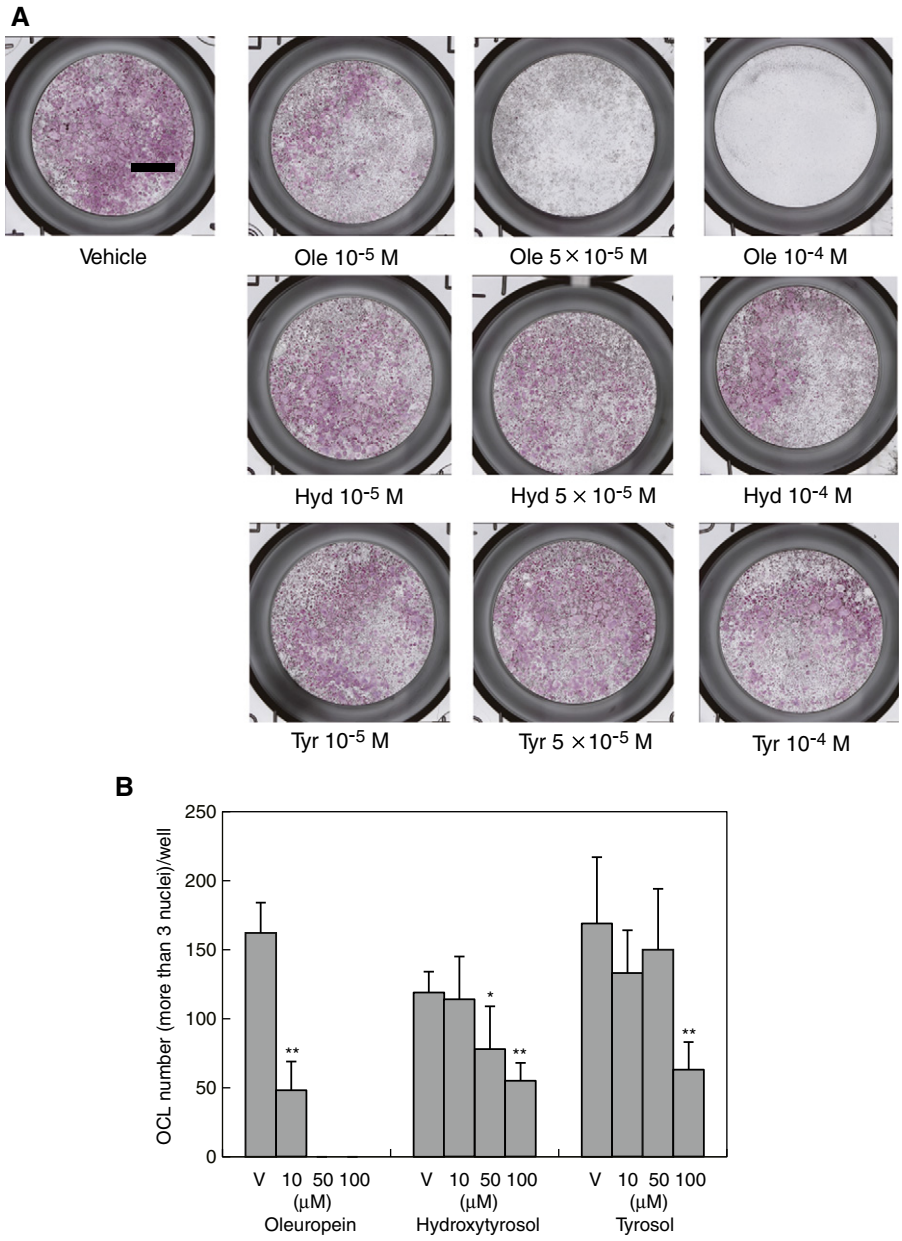

Fig. 4. Effects of olive polyphenol on formation of osteoclasts. Osteoclasts were formed from mouse spleen cells. A: Typical results of staining for detection of TRAP activity. Oleuropein (Ole), hydroxytyrosol (Hyd) or tyrosol (Tyr) was added to cultures at the indicated concentrations. Cultured cells were then stained for TRAP activity at day 7. Bar = 5 mm. B: Oleuropein, hydroxytyrosol or tyrosol was added to cultures at the indicated concentrations. Cultured cells were then stained for TRAP activity at day 7. TRAP-positive multinucleated cells (three or more nuclei) were counted under a microscope. Columns and bars show means ± S.D. of the results from five wells. Data are representative of the results of three separate experiments. *$P<0.05$ vs. vehicle (V), **$P<0.01$ vs. vehicle (V).

other hand, H_2O_2 stimulates the formation of osteoclasts (Baek et al., 2010; Bai et al., 2005; Suda et al., 1993). Thus, the effects of hydroxytyrosol on osteoblasts, osteoclasts and bone in this study may be due to diminished H_2O_2 levels in cells.

The in vivo effects of hydroxytyrosol were more potent than those of oleuropein when these compounds were orally administered to mice, although oleuropein most strongly inhibited the formation of osteoclasts and enhanced deposition of calcium by osteoblasts in culture. Bai et al. (1998) reported that synthetic hydroxytyrosol is absorbed into plasma very rapidly (0.89 to 3.26 μg/ml at 10 min) after oral administration of 10 mg hydroxytyrosol to rats. In contrast, Edgecombe et al. (2000) showed that oleuropein was poorly absorbed from isolated perfused rat intestine. Therefore, hydroxytyrosol can effectively rescue bone loss in OVX mice, as hydroxytyrosol is absorbed more readily by the body than oleuropein.

We found in this study that oleuropein and hydroxytyrosol effectively protect against the loss of bone mineral density of trabecular bone of femur in OVX mice, and that tyrosol had no effect. To date, it has been reported that black Lucques olives (Puel et al., 2007) and olive polyphenols such as oleuropein, hydroxytyrosol and tyrosol (Puel et al., 2006, 2008) prevent bone loss in the experiments using OVX rats. As bone mineral density was monitored in total rat femurs, we believed that the observed effects of polyphenols were weak. In contrast, we separately analyzed bone mineral density of trabecular bone and cortical bone in femurs of OVX mice in the present study. Our results showed that olive polyphenols markedly reduced bone loss in trabecular bone, but had no effect on cortical bone in OVX mice. Our results also revealed that tyrosol did not affect the prevention of bone loss in OVX mice. Taken together, these results suggest that olive polyphenol can prevent bone loss in many species.

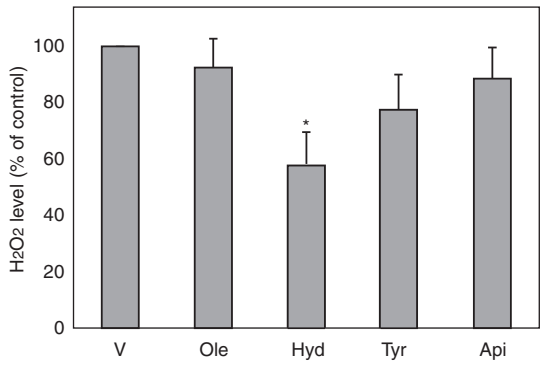

Fig. 5. Effects of olive leaf compounds on intracellular reactive oxygen species concentrations. Cells were pretreated with or without oleuropein (Ole, 50 μM), hydroxytyrosol (Hyd, 50 μM), tyrosol (Tyr, 50 μM) or apigenin (Api, 50 μM) for 48 h, followed by the incubation with 500 μM H_2O_2. Intracellular reactive oxygen species concentrations were assessed as described in Materials and methods. Data are means ± SE of three to four separate experiments. *$P<0.05$ vs. control cells (V).

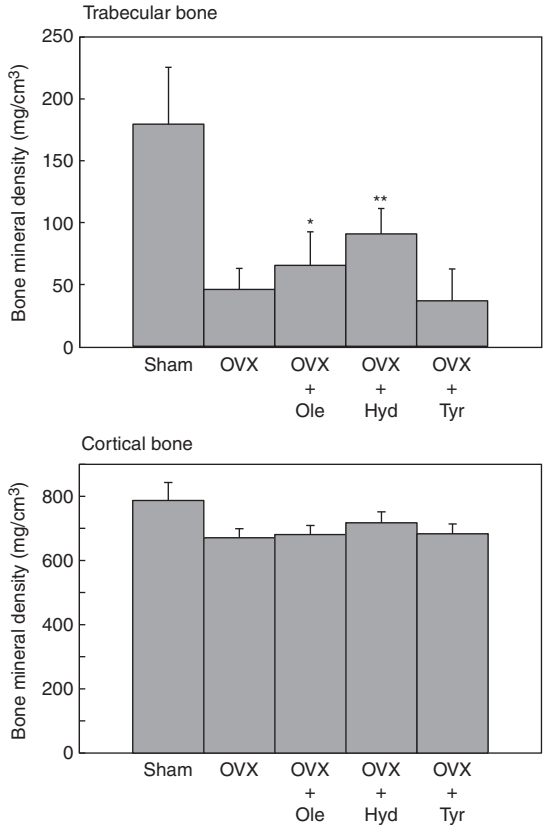

Fig. 6. Effects of oleuropein and its metabolites on bone mineral density in OVX mice. BALB/c female mice (age; 6 weeks) underwent sham operation (n = 7) or were surgically ovariectomized (OVX; n = 28) under anesthesia. Mice were randomly assigned to five groups (each n = 7): Sham, sham-operated controls; OVX controls; OVX + Ole, OVX administered orally with oleuropein (10 mg/kg body weight) at 3-day intervals; OVX + Hyd, OVX administered orally with hydroxytyrosol (10 mg/kg body weight) at 3-day intervals; and OVX + Tyr, OVX administered orally with tyrosol (10 mg/kg body weight) at 3-day intervals. Corn oil alone was orally administered to Sham and OVX control mice. After the 28-day experimental period, left and right femurs were surgically collected from anesthetized mice. Bone mineral density of femurs (trabecular bone and cortical bone) was assessed using an X-ray CT System (LA Theata LCT-100; Aloka). We monitored the bone mineral density of femurs at 0.3 mm intervals, and the values at 1.8 mm from the femur epiphysis are shown in the figures. *P<0.05 vs. OVX, **P<0.01 vs. OVX.

In conclusion, olive polyphenols, particularly hydroxytyrosol, prevented bone loss in an experimental model of osteoporosis (OVX mice). These findings suggest that olive polyphenols may provide insights into the development of tools useful in preventing and treating osteoporosis. Further investigation is required in order to clarify the detailed molecular mechanisms of action of hydroxytyrosol in bone.

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