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(-)-Epigallocatechin gallate induces apoptosis, via caspase activation, in osteoclasts differentiated from RAW 264.7 cells

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Background and Objective: Alveolar bone resorption is a characteristic feature of periodontal diseases and involves removal of both the mineral and the organic constituents of the bone matrix, a process mainly carried out by multinucleated osteoclast cells. (-)-Epigallocatechin gallate, the main constituent of green tea polyphenols, has been reported to induce the apoptotic cell death of osteoclasts and to modulate caspase activation in various tumor cells. In the present study, we investigated the inhibitory effect of (-)-epigallocatechin gallate on osteoclast survival and examined if (-)-epigallocatechin gallate mediates osteoclast apoptosis via caspase activation.

Material and Methods: The effect of (-)-epigallocatechin gallate on osteoclast survival was examined by tartrate-resistant acid phosphatase (TRAP) staining in osteoclasts differentiated from RAW 264.7 cells. In addition, we evaluated the apoptosis of osteoclasts by (-)-epigallocatechin gallate using a DNA-fragmentation assay. Involvement of caspase in (-)-epigallocatechin gallate-mediated osteoclast apoptosis was evaluated by treatment with a general caspase inhibitor, Z-VAD-FMK. Moreover, the effect of (-)-epigallocatechin gallate on the activation of caspase-3 was assessed by a colorimetric activity assay and western blotting.

Results: (-)-Epigallocatechin gallate significantly inhibited, in a dose-dependent manner, the survival of osteoclasts differentiated from RAW 264.7 cells and induced the apoptosis of osteoclasts. Treatment with (-)-epigallocatechin gallate resulted in DNA fragmentation and induced the activation of caspase-3 in RAW 264.7 cell-derived osteoclasts. Additional treatment with Z-VAD-FMK suppressed these effects of (-)-epigallocatechin gallate.

Conclusion: From these findings, we could suggest that (-)-epigallocatechin gallate might prevent alveolar bone resorption by inhibiting osteoclast survival through the caspase-mediated apoptosis.

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Bone resorption is clinically the most important issue in bone disease, such as periodontitis, because it leads to tooth loss (1). Bone resorption involves the removal of both the mineral and the organic constituents of the bone matrix. Osteoclasts, the cells principally responsible for this process (2), are the only cells that are known to have the capacity to dissolve crystallized hydroxyapatite and degrade the organic bone matrix. Osteoclasts are

bone-resorbing, multinucleated cells that are differentiated from hemopoietic progenitor cells located mainly in bone marrow (3).

Green tea is one of the most popular beverages in the world, and it has received considerable attention because of its many scientifically proven beneficial effects on human health. Several epidemiologic and experimental observations have confirmed that there is a close relationship between green tea consumption and the prevention of both cancer development and cardiovascular disease (4). These effects have been largely attributed to the most prevalent polyphenol contained in green tea, namely (-)-epigallocatechin gallate. (-)-Epigallocatechin gallate is known to induce apoptosis in various types of tumor cells, but has little or no effect on normal cells (5–7). Recently, it has been reported that (-)-epigallocatechin gallate could induce the apoptotic cell death of osteoclasts (8).

Apoptosis is a pathway of fundamental biochemical cell death, which is essential for normal tissue homeostasis, cellular differentiation and development within a multicellular organism. Apoptotic cells may be characterized by specific morphological and biochemical changes, including cell shrinkage, chromatin condensation and internucleosomal cleavage of genomic DNA (9). This fragmentation of the genomic DNA is the biochemical hallmark of apoptosis (10). Several recent studies have identified the involvement of multiple caspases in the proteolytic cascade of apoptosis in osteoclasts and different cell types (11–13). Caspases are part of a growing family of cysteine proteases, and 14 mammalian caspase sequences have been reported to date. Caspases are synthesized as inactive precursors (zymogens) that are proteolytically processed to generate active enzymes. These activating cleavage events are conducted by other caspases and are thought to represent a major regulatory step in the apoptotic pathway (11,12,14,15). Recently, (-)-epigallocatechin gallate has been shown to modulate caspase activation (16,17).

However, the precise mechanism by which (-)-epigallocatechin gallate induces apoptosis and modulates caspase activation remains to be elucidated. In addition, mechanisms of (-)-epigallocatechin gallate-mediated inhibition of osteoclast survival are incompletely understood. Such an understanding could be critical for developing (-)-epigallocatechin gallate as agents for the prevention and therapy of bone disease, such as periodontitis.

Therefore, the present study was undertaken to determine the effect of (-)-epigallocatechin gallate on osteoclast survival *in vitro*. We investigated if (-)-epigallocatechin gallate mediates osteoclast apoptosis via caspase activation in osteoclastic cells differentiated from RAW 264.7 cells.

Materials and methods

Cell culture and cell viability assay

Cells of the murine monocyte/macrophage cell line, Raw 264.7, were cultured to confluence in Dulbecco's modified Eagle's medium (Gibco BRL, Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco BRL, Life Technologies) and antibiotic-antimycotic (100 units/ml of penicillin G sodium, 100 μg/ml of streptomycin sulfate and 0.25 μg/ml of amphotericin B) (Gibco BRL, Life Technologies) in 100-mm culture dishes at 37°C in an atmosphere of 5% CO_2. The cells were then detached from the culture dish by treatment with trypsin-EDTA (Gibco BRL, Life Technologies) and collected by centrifugation.

The colorimetric assay utilizing MTT dye (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (Sigma Chemical Co., St Louis, MO, USA) was used to measure the viability of cells after treatment with (-)-epigallocatechin gallate. The number of viable cells was determined by measuring the reduction of MTT dye (by mitochondrial dehydrogenase) in live cells to blue formazan crystals (18). Raw 264.7 cells (2×10^3 per well) were seeded in 48-well plates and grown in 400 μl of Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Twenty-four hours after seeding, the cells were treated with various concentrations of (-)-epigallocatechin gallate (Calbiochem, La Jolla, CA, USA) for an additional 5 d. Subsequently, 100 μl of MTT solution (5 mg/ml) was added to each well and the cells were incubated for 4 h at 37°C. The supernatant was discarded and 400 μl of dimethylsulfoxide was added to each well to dissolve the formazan crystals. The optical density of the formazan solution was measured at 570 nm.

In vitro osteoclast differentiation

Raw 264.7 cells were seeded in 48-well plates at a density of 2×10^3 cells/well [for tartrate-resistant acid phosphatase (TRAP) staining], or in 60-mm culture dishes at a density of 6×10^3 cells/dish (for the DNA-fragmentation assay, the caspase activity assay and western blot analysis), in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. After 1 d of incubation at 37°C in an atmosphere of 5% CO_2, the differentiation of osteoclasts from RAW 264.7 cells was induced with either 50 ng/ml of recombinant mouse soluble receptor activator of nuclear factor-κB ligand (RANKL; Koma Biotech Inc., Seoul, Korea) for TRAP staining, or 50 ng/ml of RANKL + 50 ng/ml of recombinant mouse macrophage-colony stimulating factor (Koma Biotech Inc.) for the DNA-fragmentation assay, caspase activity assay and western blot analysis.

TRAP staining

Concomitant with RANKL, RAW 264.7 cells were treated with various concentrations of (-)-epigallocatechin gallate for 5 d. In addition, to examine whether caspase TS was involved in the action of (-)-epigallocatechin gallate, the cell cultures were treated with (-)-epigallocatechin gallate and/or the general caspase inhibitor, Z-VAD-FMK (R & D Systems, Inc., Minneapolis, MN, USA), as follows: (i) no treatment; (ii) Z-VAD-FMK (10 μM and 50 μM); (iii) (-)-epigallocatechin gallate (50 μM); or (iv) (-)-epigallocatechin gallate (50 μM) + Z-VAD-

FMK (10 μM and 50 μM). After treatment, the cells were fixed and stained for TRAP, an enzyme generally accepted as a marker for osteoclasts (19), using an acid phosphatase kit (Sigma Chemical Co.). TRAP-positive multinucleated cells showing more than three nuclei were considered to be osteoclasts and were counted as such.

DNA-fragmentation assay

After 4 d of incubation to allow the differentiation of osteoclasts from RAW 264.7 cells by RANKL and macrophage-colony stimulating factor, the cell cultures were treated in the presence or absence of RANKL for 24 h, as follows: (i) no treatment; (ii) Z-VAD-FMK (50 μM); (iii) (-)-epigallocatechin gallate (50 μM); or (iv) (-)-epigallocatechin gallate (50 μM) + Z-VAD-FMK (50 μM). After treatment, the TACS™ apoptotic DNA laddering kit (R & D Systems, Inc.) was used to assay for apoptosis by detecting internucleosomal DNA fragmentation and displaying DNA laddering. Briefly, after washing with cold phosphate-buffered saline (pH 7.2), the DNA was isolated by lysing the cells and purified by organic extraction and isopropanol precipitation. Precipitated DNA was pelleted by centrifugation (12,000 ***g***, 10 min at 4°C), washed with 70% ethanol, dried and dissolved in DNase-free water. The purified DNA was quantified using a spectrophotometer and resolved by electrophoresis through 1.5% TreviGel 500 gels included in the kit. The gel was stained with ethidium bromide and observed under ultraviolet illumination.

Caspase activity assay

After 4 d of incubation to allow differentiation of osteoclasts from RAW 264.7 cells by RANKL and macrophage-colony stimulating factor, the cell cultures were treated in the presence of RANKL for 24 h, as follows: (i) no treatment; (ii) Z-VAD-FMK (50 μM); (iii) (-)-epigallocatechin gallate (50 μM); or (iv) (-)-epigallocatechin gallate (50 μM) + Z-VAD-FMK (50 μM). Caspase-3 activity in cells was measured using the caspase-3 colorimetric activity assay kit (Chemicon International Inc., Temecula, CA, USA), according to the manufacturer's protocol. Briefly, the cells were washed with cold phosphate-buffered saline and lysed with cell lysis buffer included in the kit. The cell lysates were centrifuged (10,000 *g* for 5 min at 4°C), and the supernatants were collected. Equal amounts of protein (100 μg), 30 μg of colorimetric caspase-3 substrate (Ac-DEVD-pNA) and assay buffer were added to each reaction mix, which were then incubated for 2 h at 37°C. Caspase activity was determined by measuring the absorbance at 405 nm.

Western blot analysis

Cultures of RAW 264.7 cells were treated, as detailed above, for the caspase activity assay. Then, the cells were washed with cold phosphate-buffered saline and scraped into cell lysis buffer (Cell Signaling Technology, Inc., Danvers, MA, USA) after incubation on ice for 5 min. The lysate was cleared by centrifugation at 14,000 *g* for 10 min at 4°C, and the supernatant was retained for Western blotting. The protein concentration was determined by the Bradford assay (Bio-Rad, Richmond, CA, USA). Equal amounts of protein (25–50 μg) in each sample were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis in 4–12% NuPAGE Bis-Tris gels (Invitrogen, Life Technologies, Carlsbad, CA, USA) and blotted onto nitrocellulose membrane. After blocking with blocking buffer (Invitrogen, Life Technologies), the blotted membrane was incubated with purified anticaspase-3 immunoglobulin (1 : 1000 dilution; Cell Signaling Technology, Inc.) or anti-actin immunoglobulin (1 : 100 dilution; Sigma Chemical Co.) overnight at 4°C. This was followed by incubation with alkaline phosphatase-conjugated secondary antirabbit immunoglobulin (1 : 5000 dilution; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA), and protein expression was detected by chemiluminescence using a WesternBreeze Chemiluminescent kit (Invitrogen, Life Technologies).

Statistical analysis

Data were expressed as mean values ± standard deviation. Statistical differences were determined by analysis of variance using the the SAS 8.02 program. Tukey's test was used for the *post hoc* comparison of specific groups. Statistical significance was determined at the $p < 0.05$ level.

Results

Effect of (-)-epigallocatechin gallate on the viability of RAW 264.7 cells

The MTT assay was performed to assess the effect of (-)-epigallocatechin gallate on the viability of RAW 264.7 cells and to determine the appropriate concentration for treating cell cultures. Compared with nontreated cells, concentrations of up to 50 μM (-)-epigallocatechin gallate showed no effect, after 5 d of treatment, on the viability of RAW 264.7 cells. However, inhibition of cell growth was observed at the higher concentration of (-)-epigallocatechin gallate (100 μM) (Fig. 1). Therefore, (-)-epigallocatechin gallate was used at a concentration of less than 50 μM in the subsequent studies.

Effect of (-)-epigallocatechin gallate and Z-VAD-FMK on the survival of RAW 264.7 cell-derived osteoclasts

To examine the effect of (-)-epigallocatechin gallate on osteoclast survival, osteoclasts were differentiated from RAW 264.7 cell by RANKL and treated with various concentrations of (-)-epigallocatechin gallate. Treatment with (-)-epigallocatechin gallate resulted in a significant decrease in the number of TRAP-positive osteoclasts in a dose-dependent manner (Fig. 2). In addition, to examine whether caspase is involved in the effect of (-)-epigallocatechin gallate, the cell cultures were treated with (-)-epigallocatechin gallate and/or Z-VAD-FMK. Z-VAD-FMK prevented an (-)-epigallocatechin gallate-induced decrease in the number of osteoclasts. This effect was more prominent at 50 μM Z-VAD-FMK than at 20 μM. However, Z-VAD-FMK itself had no effect on

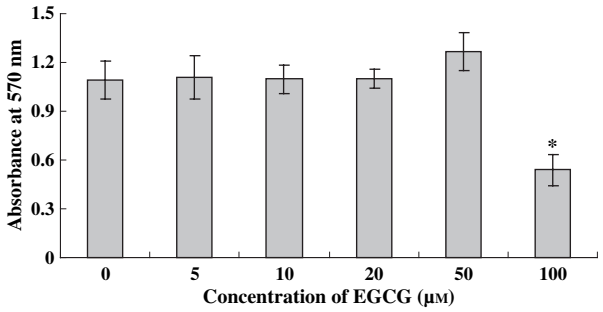

Fig. 1. The effect of (-)-epigallocatechin gallate on the viability of RAW 264.7 cells. RAW 264.7 cells were treated with the indicated concentrations of (-)-epigallocatechin gallate for 5 d. The cellular activity was then estimated by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, and the results are expressed as the mean ± standard deviation of six cultures. The data are representative of three separate experiments. *$p < 0.05$, significantly different from the nontreated group. EGCG, (-)-epigallocatechin gallate.

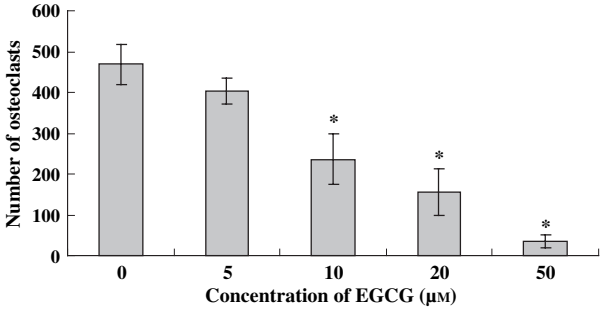

Fig. 2. Inhibitory effect of (-)-epigallocatechin gallate on the survival of RAW 264.7 cell-derived osteoclasts. Osteoclasts were differentiated from RAW 264.7 cells by nuclear factor-κB ligand (RANKL) and treated with the indicated concentrations of (-)-epigallocatechin gallate. Tartrate-resistant acid phosphate (TRAP)-positive multinucleated cells with more than three nuclei were counted as osteoclasts. The results are expressed as the mean ± standard deviation of five cultures. The data are representative of three separate experiments. *$p < 0.05$, significantly different from the nontreated group. EGCG, (-)-epigallocatechin gallate.

the number of TRAP-positive osteoclasts, compared with the nontreated cells (Fig. 3).

(-)-Epigallocatechin gallate-induced apoptosis in RAW 264.7 cell-derived osteoclasts

To investigate whether treatment with (-)-epigallocatechin gallate induced apoptosis in RAW 264.7 cell-derived osteoclasts, cells were treated with (-)-epigallocatechin gallate (50 μM) in the presence or absence of RANKL (50 ng/ml) for 24 h. In each case, nucleosomal DNA fragmentation, which is typical of apoptosis, was visible and the fragmentation pattern was most prominent in cells treated with (-)-epigallocatechin gallate in the absence of RANKL. This result shows that the presence of RANKL in the culture medium slightly sustains the survival of osteoclasts. In addition, 50 μM Z-VAD-FMK reduced the extent of DNA fragmentation and significantly blocked (-)-epigallocatechin gallate from inducing apoptosis (Fig. 4).

(-)-Epigallocatechin gallate-induced activation of caspase-3 in RAW 264.7 cell-derived osteoclasts

Another feature of apoptotic cell death – the activation of caspase-3 – was evaluated by western blotting and the colorimetric activity assay of caspase-3. RAW 264.7 cell-derived osteoclasts were treated with (-)-epigallocatechin gallate (50 μM) in the presence of RANKL (50 ng/ml) for 24 h. In western blotting, the antibody used recognizes the intact 35-kDa proform and the cleaved 17-kDa active form of caspase-3. The treatment with (-)-epigallocatechin gallate (50 μM) increased the amount of the cleaved 17-kDa fragment of caspase-3 and decreased the amount of the 35-kDa pro-caspase-3, indicating the activation of caspase-3, compared with untreated control cells (Fig. 5). In addition, 50 μM Z-VAD-FMK reduced the intensity of the active form of caspase-3 and blocked the activation of caspase-3 by (-)-epigallocatechin gallate (Fig. 5). The activity of caspase-3 in (-)-epigallocatechin gallate-treated and -untreated RAW 264.7 cell-derived osteoclasts was also measured. The cells treated with (-)-epigallocatechin gallate showed a significantly higher activity of caspase-3, which was blocked by Z-VAD-FMK (Fig. 6).

These results indicate that (-)-epigallocatechin gallate induces the activation of caspase-3, which is associated with reduced cell survival and apoptosis of (-)-epigallocatechin gallate-treated osteoclasts, as shown in Fig. 3.

Discussion

Recently, green tea has been focused on as a result of numerous biological effects of its constituents (4). Green tea consists mainly of polyphenols (catechins), which constitute up to 30% of the dry weight. Among these polyphenols, (-)-epigallocatechin gallate is the most abundant catechin and the one that has been the most extensively studied (20). Osteoclasts are bone-resorbing multinucleated cells. The bone-resorbing activity of osteoclasts is significantly increased in the pathogenesis of bone disease such as periodontitis.

In our recent study, we reported that 20 μM (-)-epigallocatechin gallate significantly inhibited osteoclast formation in the coculture system of bone marrow cells and primary osteoblastic cells. However, the precise mechanism

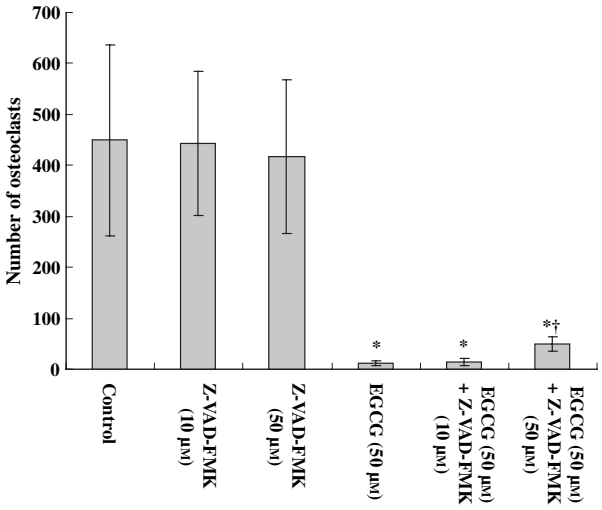

Fig. 3. General caspase inhibitor (Z-VAD-FMK) blocked the effect of (-)-epigallocatechin gallate on the survival of RAW 264.7 cell-derived osteoclasts. The cell cultures were treated with (-)-epigallocatechin gallate and/or Z-VAD-FMK to examine whether caspase is involved in the action of (-)-epigallocatechin gallate. Tartrate-resistant acid phosphate (TRAP)-positive multinucleated cells with more than three nuclei were counted as osteoclasts. The results are expressed as the mean ± standard deviation of six cultures. The data are representative of three separate experiments. *$p < 0.05$, significantly different from the nontreated control group; †$p < 0.05$, significantly different from the (-)-epigallocatechin gallate-treated group. EGCG, (-)-epigallocatechin gallate.

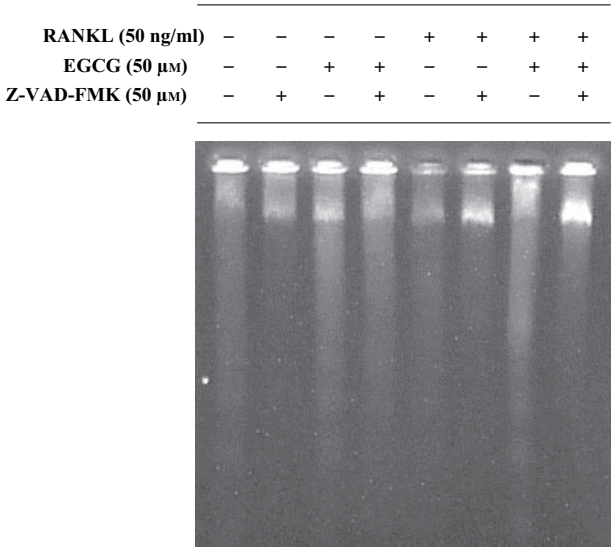

Fig. 4. (-)-Epigallocatechin gallate-induced nucleosomal DNA fragmentation in RAW 264.7 cell-derived osteoclasts. Cells were incubated with (-)-epigallocatechin gallate or the general caspase inhibitor, Z-VAD-FMK, for 24 h in the presence or absence of nuclear factor-κB ligand. The data are representative of duplicate experiments. EGCG, (-)-epigallocatechin gallate; RANKL, nuclear factor-κB ligand.

of this effect was not established (21). Some studies have shown that apoptosis is the mechanism of action of (-)-epigallocatechin gallate for inhibiting cell growth in various type of tumor cells (5–7). Thus, in the present study, we re-evaluated the effects of (-)-epigallocatechin gallate on osteoclasts differentiated from RAW 264.7 cells and investigated if caspase-mediated apoptosis is involved in the effect of (-)-epigallocatechin gallate on osteoclasts. Our present findings show clearly that (-)-epigallocatechin gallate was highly effective in inhibiting the survival of RAW 264.7 cell-derived osteoclasts *in vitro* (Fig. 2) and demonstrate that this inhibition might be mediated, in part, through the apoptosis of osteoclasts by the formation of a DNA-fragmentation pattern, the most significant feature of apoptosis (Fig. 4).

In addition, these inhibitory effects of (-)-epigallocatechin gallate on osteoclasts were mildly suppressed by Z-VAD-FMK, suggesting the partial involvement of caspase in the (-)-epigallocatechin gallate-induced apoptosis of RAW 264.7 cell-derived osteoclasts (Fig. 3). This finding is supported by the present results that (-)-epigallocatechin gallate stimulates the activation of caspase-3, the elevation of caspase-3 activity and the cleavage of pro-caspase-3 (Figs 5 and 6). It is known that pro-caspase-3 (35 kDa) is cleaved to yield 17- and 12-kDa fragments when activated. In addition, caspase-3, defined as a key executioner of apoptosis, is the most prevalent caspase in the cell. Moreover, it is known that caspase-3 is the caspase ultimately responsible for the majority of the apoptotic effect, although it is supported by caspase-6 and -7 (12). We also found that this (-)-epigallocatechin gallate-induced activation of caspase-3 was suppressed by Z-VAD-FMK (Figs 5 and 6). Therefore, our results suggest that the induction of apoptosis in RAW-264.7 cell-derived osteoclasts involves the activation of caspase, in particular, caspase-3.

However, although Z-VAD-FMK strongly suppressed the (-)-epigallocatechin gallate-induced activation of caspase-3, it only partially suppressed (-)-epigallocatechin gallate-induced apoptosis (Fig. 4). In addition, Z-VAD-FMK could not completely restore the decrease in the number of osteoclasts by (-)-epigallocatechin gallate (Fig. 3). This suggests that mechanisms using different signaling pathways could be involved in the (-)-epigallocatechin gallate-induced apoptosis of osteoclasts as well as the caspase pathway. In this respect, numerous studies have shown

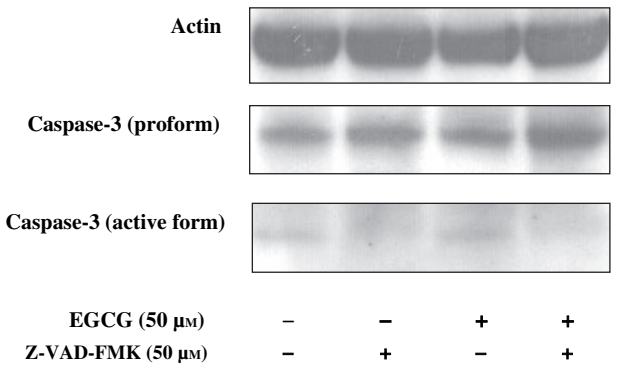

Fig. 5. The effect of (-)-epigallocatechin gallate on the activation of caspase-3 in RAW 264.7 cell-derived osteoclasts. Cells were treated with (-)-epigallocatechin gallate or with the general caspase inhibitor, Z-VAD-FMK, for 24 h. Western blotting analysis was performed using anticaspase-3 immunoglobulin that recognizes the intact proform (32 kDa) and cleaved active form (17 kDa) of caspase-3. Actin served as an endocontrol. The data are representative of duplicate experiments. EGCG, (-)-epigallocatechin gallate.

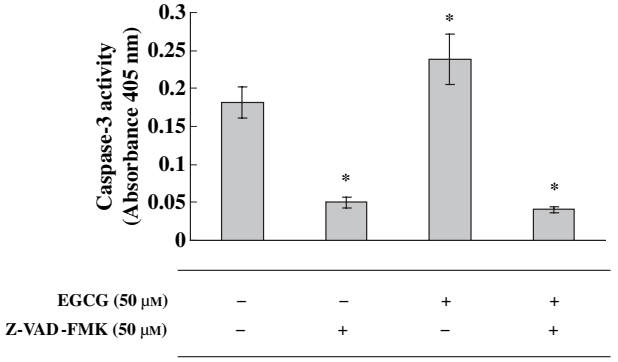

Fig. 6. The effect of (-)-epigallocatechin gallate on the activity of caspase-3 in RAW 264.7 cell-derived osteoclasts. Cells were treated with (-)-epigallocatechin gallate or with the general caspase inhibitor, Z-VAD-FMK, for 24 h. The results are expressed as the mean ± standard deviation of four cultures. The data are representative of duplicate experiments. $*p < 0.05$, significantly different from the nontreated group. EGCG, (-)-epigallocatechin gallate.

that the transcription factor, nuclear factor-κB, regulates the susceptibility of cells to apoptosis through the transcriptional control of genes. Nuclear factor-κB plays a critical role in the regulation of genes related to cell survival, proliferation and apoptosis (22–24). In a previous study, it has been reported that (-)-epigallocatechin gallate is capable of inducing apoptosis by inhibiting nuclear factor-κB activation in cancer cells (22,25). However, it remains to be determined which pathway is more important in the apoptosis of osteoclasts by (-)-epigallocatechin gallate.

In addition, the precise mechanism of caspase activation in (-)-epigallocatechin gallate-induced apoptosis has not yet been fully established. It was suggested that the binding of (-)-epigallocatechin gallate to Fas, presumably on the cell surface, triggers the Fas-mediated apoptosis in tumor cells (17). Moreover, it was reported that Fas-mediated caspase activation is involved in apoptosis in osteoclasts (26).

In previous studies, osteoclasts are reported to have a short half-life. They undergo apoptosis *in vitro* within a few days (27). It was also reported that the absolute number of osteoclasts undergoing apoptosis, at any given time, ranges from 10 to 80% (28), and more than 80% of the purified osteoclasts were found to undergo apoptotic cell death by 48 h during culture (13). Similarly, in our experiment, untreated control cells showed a relatively high level of the apoptosis and caspase-3 activity (Figs 4–6). In addition, the number of osteoclasts is dependent upon the relative rates of osteoclastogenesis and apoptosis. Although we showed that (-)-epigallocatechin gallate induced the apoptosis of osteoclasts, we cannot exclude the possibility that (-)-epigallocatechin gallate plays a role in the regulation of osteoclast formation.

On the other hand, (-)-epigallocatechin gallate had no effect on RAW 264.7 cells themselves. This result suggests that (-)-epigallocatechin gallate has an effect on differentiated osteoclasts but not the undifferentiated cells. Several studies have indicated that tumor cells are much more sensitive to apoptosis induction by (-)-epigallocatechin gallate than the normal counterparts (5–7). In our previous study, we also showed that (-)-epigallocatechin gallate had no inhibitory effect on the cell viability of either coculture system or primary osteoblastic cells at concentrations of up to 20 μM. Taken together, these results show that (-)-epigallocatechin gallate, at an appropriate dose, inhibits osteoclasts but not other osteogenic cells. Therefore, (-)-epigallocatechin gallate could have an advantage as a chemopreventive agent specific for osteoclasts.

In this study, we showed that treatment of RAW 264.7 cell-derived osteoclasts with (-)-epigallocatechin gallate significantly lowered the number of osteoclasts and there was evidence of the induction of apoptosis, as shown by DNA fragmentation and partial involvement of caspase-3. In addition, the ability of Z-VAD-FMK to block apoptosis shows the caspase-dependent property of (-)-epigallocatechin gallate-induced apoptosis. Our results also show that the treatment of osteoclasts with (-)-epigallocatechin gallate significantly activated caspase-3, compared with the controls. Thus, our data revealed a novel finding that the (-)-epigallocatechin gallate-induced apoptosis in RAW 264.7 cell-derived osteoclasts was mediated, in part, through the activation of caspase-3.

From these findings, we suggest that (-)-epigallocatechin gallate might prevent alveolar bone resorption, which occurs in periodontal diseases, by inhibiting osteoclast survival through caspase-mediated apoptosis. Therefore, our results indicate that the inhibitory activity of (-)-epigallocatechin gallate could be utilized in the development of a therapeutic agent for the treatment of bone diseases, such as periodontitis. However, because we examined only the *in vitro* effects of (-)-epigallocatechin gallate in the present study, it remains to be determined whether (-)-epigallocatechin gallate also exerts these effects *in vivo*.

Acknowledgements

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