Dexamethasone inhibits bone resorption by indirectly inducing apoptosis of the bone-resorbing osteoclasts via the action of osteoblastic cells

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Abstract

Although glucocorticoids (GCs) are physiologically essential for bone metabolism, it is generally accepted that high doses of GCs cause bone loss through a combination of decreased bone formation and increased bone resorption. However, the action of GCs on mature osteoclasts remains contradictory. In this study, we have examined the effect of GCs on osteoclastic bone-resorbing activity and osteoclast apoptosis, by using two different cell types, rabbit unfractionated bone cells and highly enriched mature osteoclasts (>95% of purity). Dexamethasone (Dex, 10^{-10} – 10^{-7} M) inhibited resorption pit formation on a dentine slice by the unfractionated bone cells in a dose- and time-dependent manner. However, Dex had no effect on the bone-resorbing activity of the isolated mature osteoclasts. When the isolated osteoclasts were co-cultured with rabbit osteoblastic cells, the osteoclastic bone resorption decreased in response to Dex, dependent on the number of osteoblastic cells. Like the effect on the bone resorption, Dex induced osteoclast apoptosis in cultures of the unfractionated bone cells, whereas it did not promote the apoptosis of the isolated osteoclasts. An inhibitor of caspases, Z-Asp-CH₂-DCB attenuated both the inhibitory effect on osteoclastic bone resorption and the stimulatory effect on the osteoclast apoptosis. In addition, the osteoblastic cells were required for the osteoclast apoptosis induced by Dex. These findings indicate that the main target cells of GCs are non-osteoclastic cells such as osteoblasts and that GCs indirectly inhibit bone resorption by inducing apoptosis of the mature osteoclasts through the action of non-osteoclastic cells. This study expands our knowledge about the multifunctional roles of GCs in bone metabolism.

Abbreviations: GCs, glucocorticoids; Dex, dexamethasone; TRAP, tartrate-resistant acid phosphatase; MNCs, multinucleate cells; ODF/OPGL/TRANSE/RANKL, osteoclast differentiation factor/osteoprotegerin ligand/TNF-related activation-induced cytokine/receptor activator of NF- κ B ligand; OCIF, osteoclastogenesis inhibitory factor.

Introduction

Glucocorticoids (GCs) have diverse and complex effects on bone metabolism. When pharmacological doses of GCs were introduced for therapeutic use because of their anti-inflammatory and immunosuppressive effects, it became clear that the patients exhibited a severe osteoporosis (Lukert and Kream, 1996). This GC-induced osteoporosis was also reported in other vertebrates including dogs, rats, and mice (Altman et al., 1992; Quarles 1992; Turner et al., 1995).

In contrast, GC treatment of rats increased the bone mass, probably by inhibiting bone resorption (Goulding and Gold 1988; King et al., 1996). Like *in vivo* studies, many *in vitro* studies have shown contradictory effects of GCs on bone formation and resorption. Physiological concentrations of GCs transiently stimulated collagen synthesis in fetal rat calvariae, the stimulation being dependent on the activity of endogenous insulin-like growth factor (IGF)-I (Dietrich et al., 1979; Kream et al., 1990). In addition, in the same dose range, GCs enhanced the formation of bone

nodules in cultures of primary osteoblasts and bone marrow cells (Bellows et al., 1990; Shalhoub et al., 1992). However, higher concentrations of GCs inhibited proliferation and function of cells of the osteoblast lineage (Ng et al., 1989; McCarthy et al., 1990; Doherty et al., 1995; Weinstein et al., 1998). Thus, the effects of GCs depend on the concentrations and further on the stage of differentiation of the cells in the experimental models. Likewise, many experimental studies have yielded controversial actions of GCs on bone resorption, as there have been reported both inhibitory and stimulatory effects on ^{45}Ca release from bones in organ cultures of rat long bones and of rat and mouse calvariae, respectively (Raisz et al., 1972; Reid et al., 1986; Gronowicz et al., 1990). Regarding osteoclastogenesis, the stimulatory effect of GCs was reported in culture of bone marrow cells (Udagawa et al., 1989; Shuto et al., 1994), whereas GCs inhibited osteoclastic bone resorption due to a cytotoxic action (Tobias and Chambers, 1989; Dempster et al., 1997). However, it was difficult to define the target cells of GCs in those culture systems because of the heterogeneity of the cells.

We previously developed a new method for isolation of functional osteoclasts with an extremely high purity (more than 95%) from rabbit bones (Kakudo et al., 1996). Thereby, direct actions of osteotropic hormones and local factors on mature osteoclasts *in vitro* could be precisely estimated without the influence of non-osteoclastic cells such as stromal and osteoblastic cells. For example, direct inhibition by estrogen and stimulation by Gas6 of osteoclastic bone resorption were reported (Kameda et al., 1997; Nakamura et al., 1998). In addition, by use of co-cultures of isolated osteoclasts and other bone cells, we are able to evaluate the indirect action of the agonists and antagonists on the osteoclastic bone resorption. In this study, using osteoclasts highly enriched by the method mentioned above and unfractionated bone cells, we examined whether GCs directly or indirectly act on osteoclast function. Here we report our findings indicating that GCs indirectly inhibit the bone-resorbing activity by acting on osteoblastic cells and that the inhibitory effect is mediated by osteoclast apoptosis.

Materials and methods

Materials

Six-day-old Japan white rabbits were purchased from

Japan Laboratory Animals, Inc. (Tokyo, Japan). Dexamethasone (Dex) was obtained from Sigma (St. Louis, MO, USA). Collagen gel solution (type-I collagen, cell matrix type I-A) and pronase E were obtained from Nitta Gelatin Co. (Osaka, Japan) and Kaken Chemical Co. (Tokyo, Japan), respectively. The collagen gel solution was prepared from porcine tendons and solubilized in an acidic solution. Bacterial collagenase, Hoechst 33258 and glutaraldehyde were purchased from Wako Pure Chemical Co. (Osaka, Japan). Carbobenzoxycarbonyl-L-aspartyl-[(2,6-dichlorobenzoyl)oxy]methane (Z-Asp-CH₂-DCB), an inhibitor of caspases, was obtained from Peptide Institute, Inc., Osaka, Japan. Leukocyte acid phosphatase staining kit was from Sigma Chemical Co. (St. Louis, MO, USA), and alpha-modified minimum essential medium (α -MEM) and fetal bovine serum (FBS) were from Flow Laboratories (McLean, VA, USA) and Filttron Pty, Ltd. (Brooklyn, Australia). Tissue culture dishes, type-I collagen-coated dishes and 96-well culture plates were purchased from Becton Dickinson (Lincoln Park, NJ, USA).

Preparation of rabbit unfractionated bone cells and isolation of mature osteoclasts in suspension from collagen gels

Long bones were obtained from six-day-old rabbits, and soft tissues attached to the bone were removed. The bones were minced in α -MEM containing 5% FBS. The cells were dissociated from the bone fragments by vortexing, and the fragments were then allowed to sediment for 1 min. The cells in the supernatant were collected and used as unfractionated bone cells. Rabbit osteoclasts were isolated from collagen gels by the method of Kakudo et al. (1996). Briefly, 2×10^8 unfractionated bone cells prepared as mentioned above were plated onto a 0.24% collagen gel in each of several 100-mm tissue culture dishes and incubated for 2 h at 37 °C in a 5% CO₂ incubator. The cultures were then treated with 0.001% pronase E and 0.02% EDTA for 10 min, washed three times with phosphate-buffered saline (PBS), and treated with 0.01% bacterial collagenase for 5 min at room temperature to remove cells other than osteoclasts. After several washings, the cultures were finally digested with 0.1% collagenase for 10 min to free the remaining cells (osteoclasts) from the gel. The released cells were collected by centrifugation and suspended in α -MEM containing 5% FBS. More than 95% of the cells thus prepared were tartrate-resistant

Figure 1. Photomicrographs of purified osteoclasts and apoptotic osteoclasts. Mature osteoclasts purified and harvested from collagen gels were replated and incubated for 1 h in type-I collagen-coated dishes and then stained for TRAP activity (A), or incubated for 12 h on dentine slices (B). Unfractionated bone cells were treated with Dex (10^{-7} M) for 24 h, and then stained with Hoechst 33258; C indicates the intact (arrow) and apoptotic (arrowhead) osteoclastic MNCs, respectively, in the culture. The apoptotic MNCs display chromatin condensation. (A) Phase-contrast micrograph. (B) Scanning electron micrograph. (C) Fluorescence micrograph. Bar indicates 100 μ m.

acid phosphatase (TRAP) -positive and multinucleate with more than three nuclei (Figure 1A). We used these cells as mature osteoclasts.

Bone resorption assay

Bone resorption assays were done by the method of Takada et al. (1992) with minor modification. Unfractionated bone cells (4×10^5) or isolated osteoclasts (2×10^2) were plated and cultured on a dentine slice

(6 mm in diameter) in α -MEM supplemented with 5% FBS in each well of 96-well plates. After a 2-h incubation, the medium was replaced with α -MEM containing 5% FBS with or without Dex and/or Z-Asp-CH2-DCB. After having been cultured for the desired periods at 37 °C in a 10% CO₂ incubator, the cells were scraped off the dentine slices, and the slices were then stained with acid hematoxylin (Sigma Chemicals, St. Louis, MO, USA). The stained pit areas were determined under a microscope at $\times 100$ magnification by counting the number of mesh squares (100 $\times$ 100 μ m) covering the pit to evaluate possible osteoclastic bone resorption. In the case of TRAP staining, at the end of the culture on dentine slices, the osteoclasts were fixed with formalin-acetone solution (37% formalin: acetone: 18 mM citric acid/19 mM sodium citrate/12 mM NaCl; 7:65:25) and stained for TRAP activity with a leukocyte acid phosphatase kit (Sigma).

Culture of osteoblastic cells

After preparing rabbit unfractionated bone cells as described above, we subsequently digested the bone fragments five times with 0.1% bacterial collagenase in PBS for 20 min each time at 37 °C. Cells released from the bone fragments between the third and fifth digestion were collected, and cultured in α -MEM with 10% FBS in 100-mm dishes until subconfluent. The cells were digested, and stored at -80 °C until used.

Osteoclast apoptosis

The fluorescence microscopic method used to detect osteoclast apoptosis was described previously (Kameda et al., 1997). Unfractionated bone cells were incubated for 3 h in α -MEM containing 5% FBS in 60-mm dishes. Then, the cells were treated with or without various concentrations of Dex and/or A-asip-CH2-DCB for the indicated times. At the end of the treatment, the cells were washed to remove non-adherent cells, and the adherent cells were fixed in 2% glutaraldehyde solution for 10 min. For evaluation of the direct effect of Dex on the apoptosis of osteoclasts, the osteoclasts isolated from collagen gels as mentioned above were resuspended in α -MEM containing 5% FBS, seeded onto type-I collagen-coated plastic dishes, incubated with various doses of Dex in α -MEM containing 5% FBS, and then fixed. The fixed cells were stained with 0.2 mM Hoechst 33258 for visualization of chromatin condensation under a

fluorescence microscope (VANOX AHB-LB, Olympus Co., Tokyo, Japan). Then, numbers of total MNCs and apoptotic MNCs showing chromatin condensation were counted.

Statistical analysis

Intergroup differences in mean values were statistically analyzed by Student's *t*-test.

Results

The osteoclastic bone resorption in response to Dex treatment was first determined in cultures of rabbit unfractionated bone cells (Figure 2). The addition of Dex decreased pit area excavated by osteoclasts in a dose-dependent manner, and the inhibitory effect was significant at 10^{-10} – 10^{-7} M. The pit area at 10^{-9} M Dex decreased to about 40% of the control in 24-h cultures. In contrast, the number of TRAP-positive MNCs on a dentine slice was not altered by the Dex addition. The inhibition of the osteoclastic bone resorption was observed as early as 12 h after Dex addition, and reached a maximal at 24 h, but thereafter the rate of the inhibition decreased. The culture of unfractionated bone cells contained a variety of cell types including bone marrow stromal cells, osteoblasts and hematopoietic cells as well as osteoclasts. Therefore, using a highly purified population of rabbit osteoclasts, we next examined the direct effect of Dex on the bone-resorbing activity of osteoclasts. As shown in Figure 1A, the purity of isolated osteoclasts in the culture was more than 95% based on the number of TRAP-positive MNCs. In addition, the isolated osteoclasts alone could resorb dentine even without any support from bone marrow stromal cells or osteoblastic cells (Figure 1B). As shown in Figure 3, when the isolated osteoclasts were incubated with Dex on dentine slices for 18 h, the bone-resorbing activity and the number of the cells on dentine slices did not change throughout the Dex treatment (up to 72 h). These results suggest that the inhibition of osteoclastic bone resorption elicited by Dex is mediated by Dex-action on cells other than osteoclasts. To assess this possibility, we examined the effect of Dex on osteoclastic bone resorption in co-cultures with isolated osteoclasts and osteoblastic cells. Although the bone-resorbing activity of the isolated osteoclasts was unchanged by Dex addition, as shown in Figure 3, when the osteoclasts were co-cultured with

osteoblastic cells, Dex decreased the bone-resorbing activity dependent on the number of the osteoblastic cells added to the cultures (Figure 4).

Regarding the cellular mechanism, many *in vitro* studies have shown that osteoclast apoptosis induced by some agents such as bisphosphonates and estrogen results in a reduction in osteoclastic bone resorption (Hughes et al., 1995, 1996; Kameda et al., 1997). In addition, Dex is well known to induce apoptosis in a variety of cells (Stoetzer et al., 1999; Schmidt et al., 1999). Therefore, using both cultures of unfractionated bone cells and isolated osteoclasts, we next examined the effect of Dex on osteoclast apoptosis. When the unfractionated bone cells were exposed to Dex, some of osteoclastic MNCs in the cultures displayed characteristics of apoptosis including chromatin condensation and altered cellular and nuclear morphology as detected by fluorescence microscopy (Figure 1C). By counting numbers of intact and apoptotic osteoclastic MNCs, we determined the impact of Dex on osteoclast apoptosis in cultures of unfractionated bone cells (Figure 5). Basal value indicating the percentage of apoptotic osteoclastic MNCs per total MNCs was about 4%. The addition of Dex caused a time- and dose-dependent increase in the number of apoptotic osteoclastic MNCs in the cultures of unfractionated bone cells, with a maximal effect of 7-fold at 10^{-7} M. The effective dose range of Dex correlated with that for inhibition of bone resorption. The stimulatory effect on the apoptosis appeared as early as 3 h after the Dex addition, and continued for at least 24 h. When isolated osteoclasts were cultured alone, the basal level of apoptotic osteoclasts was about 15%, more than that in the cultures of unfractionated bone cells (Figure 6). However, Dex by itself did not change the number. In co-cultures of the isolated osteoclasts with osteoblastic cells, Dex induced an increase in the number of apoptotic osteoclasts. Finally, we examined the relationship between the inhibition of bone resorption and the osteoclast apoptosis induced by Dex (Figure 7). Z-Asp-CH2-DCB, an inhibitor of caspases, which enzymes play a crucial role in the induction of apoptosis, abrogated both the inhibitory effect of Dex on the osteoclastic bone resorption and the stimulatory effect on the osteoclast apoptosis in cultures of unfractionated bone cells.

Discussion

In this study, using cultures of unfractionated

Figure 2. Dose- and time-dependent effect of Dex on osteoclastic bone resorption in cultures of rabbit unfractionated bone cells. (A) Unfractionated bone cells on dentine slices were treated with various concentrations of Dex for 24 h, and then the area of pits (closed bars) excavated by unfractionated bone cells and the number of TRAP-positive MNCs on the dentine slices (open bars) were measured. (B) The cells were incubated in the absence (open circles) or presence (closed circles) of Dex (10^{-7} M) for the indicated times, and then the area of pits was measured. The experiments were performed three times, and the values shown are means $\pm$ SD for 5 cultures in a representative experiment. * $p < 0.01$, ** $p < 0.05$ vs. untreated cells.

Figure 3. Dose- and time-dependent effect of Dex on osteoclastic bone resorption in cultures of isolated rabbit osteoclasts. (A) Isolated mature osteoclasts on dentine slices were treated with various concentrations of Dex for 24 h, and then the area of pits (closed bars) excavated by the bone cells and the number of TRAP-positive MNCs on the dentine slice were measured. (B) The osteoclasts were incubated in the absence (open circles) or presence (closed circles) of Dex (10^{-7} M) for the indicated times, and then the area of pits was measured. The experiments were performed three times, and the values shown are means $\pm$ SD for 5 cultures in a representative experiment. * $p < 0.01$, ** $p < 0.05$ vs. untreated cells.

Figure 4. Osteoblastic cell-dependent effect of Dex on bone-resorbing activity of isolated rabbit osteoclasts. The indicated numbers of rabbit osteoblastic cells were cultured overnight on a dentine slice in α -MEM containing 5% FBS in each well of 96-well plates. Then, the isolated osteoclasts (150 cells) were seeded and incubated with (closed bars) or without (open bars) Dex (10^{-7} M) for 24 h. At the end of the culture time, the pit area (A) and the number of TRAP-positive MNCs (B) were measured. The experiments were performed three times, and the values indicate the means $\pm$ SD for 5 cultures in a representative experiment. * $p < 0.01$ vs. untreated cells.

Figure 5. Effect of Dex on osteoclast apoptosis in culture of unfractionated bone cells. Unfractionated bone cells in 60-mm plastic dishes were treated with various concentrations of Dex for 24 h (A) and other cells were incubated in the absence (open circles) or presence (closed circles) of Dex (10^{-7} M) for the indicated times (B). Then, the nuclei of the cells were stained, and the numbers of apoptotic MNCs and total MNCs were counted under a fluorescence microscope. The percentage of the apoptotic MNCs per the total MNCs was calculated. The experiments were performed three times, and the values shown are means $\pm$ SD for 5 cultures in a representative experiment. * $p < 0.01$ vs. untreated cells.

bone cells and highly purified mature osteoclasts, we demonstrated that Dex indirectly inhibits bone-resorbing activity of osteoclasts by acting on non-osteoclastic cells such as osteoblastic cells. Just as the indirect inhibition of osteoclastic bone resorption, the induction of osteoclast apoptosis by Dex was also dependent on the action of Dex on cells besides osteoclasts. In addition, the inhibition of bone resorption was at least in part mediated by inducing osteoclast apoptosis through non-osteoclastic cells because an inhibitor of caspases attenuated both inhibition of the bone resorption and enhancement of osteoclast apoptosis induced by Dex in the culture of unfractionated bone cells. Regarding osteoclast apoptosis, isolated mouse osteoclasts could not survive without macrophage colony-stimulating factor, interleukin-1 or osteoclast differentiation factor (ODF)/osteoprotegerin ligand (OPGL)/TNF-related activation-induced cytokine (TRANCE)/receptor activator of NF- κ B (RANK) ligand (RANKL), and immediately detached off after spontaneously undergoing apoptosis (Fuller et al., 1993; Greenfield et al., 1999; Jimi et al., 1999). Therefore, it is difficult to estimate the precise situation of the osteoclast apoptosis. However, we confirmed by video monitoring that rabbit osteoclasts did not detach easily even after the apoptosis had occurred. Therefore, the culture system of rabbit osteoclasts is a good experimental model for the study of osteoclast apoptosis.

Our findings are consistent with the previous studies indicating that GCs impaired bone-resorbing activity and viability of osteoclast disaggregated from neonatal rat long bones by inducing osteoclast apoptosis (Tobias and Chambers, 1989; Dempster et al., 1997). Those studies also demonstrated that the Dex effects on osteoclasts were direct. In contrast, in this study we showed the effects of Dex on rabbit osteoclasts were mediated by non-osteoclasts such as osteoblastic cells. Although the precise reason for this discrepancy remains unclear, it may be due to the difference in animal species. In addition, the isolated rabbit osteoclasts presented here were extremely homogeneous with more than 95% of purity, and the inhibitory effect of Dex on the osteoclastic bone resorption was quite dependent on number of osteoblastic cells. Therefore, the above difference in results may be attributed to different populations of cells in the experimental models employed.

At present, the molecular mechanisms for the effect of Dex on osteoclasts via osteoblastic cells are little known. So far, estrogen, bisphosphon-

Figure 6. Osteoblastic cell-dependent effect of Dex on apoptosis of isolated osteoclasts. The isolated osteoclasts were seeded into type-I collagen-coated 60 mm-plastic dishes containing or lacking osteoblastic cells (an initial density of 10^6 /dish) that had been previously added or not the day before, and then incubated with (closed bars) or without (open bars) Dex (10^{-7} M) for 24 h. Then, the percentage of the apoptotic MNCs was measured. The experiments were performed three times, and the values shown are means $\pm$ SD for 5 cultures in a representative experiment. * $p < 0.01$ vs. untreated cells.

ates, and transforming growth factor- β have been demonstrated to inhibit osteoclastic bone resorption and to promote osteoclast apoptosis (Hughes et al., 1995, 1996; Kameda et al., 1997). Estrogen and bisphosphonates were not present in our culture system, and transforming growth factor- β had no effect on the isolated rabbit osteoclasts (Kameda et al., 1997). ODF/OPGL/TRANCE/RANKL was recently been identified as the most important and critical molecule for osteoclast development and function (Lacey et al., 1998; Yasuda et al., 1998). Along with ODF/OPGL/TRANCE/RANKL, osteoclastogenesis inhibitory factor (OCIF)/osteoprotegerin (OPG) was also cloned as a potent inhibitor of osteoclastogenesis and osteoclastic bone resorption, which interferes with the binding of ODF/OPGL/TRANCE/RANKL to its receptor, RANK, by acting as a decoy receptor (Simonet et al., 1997; Tsuda et al., 1997). Both the stimulator and the inhibitor are produced by bone marrow stromal cells and osteoblastic cells, and the production was shown to be regulated by osteotropic hormones and local

Figure 7. Effect of an inhibitor of caspases, Z-Asp-CH₂-DCB, on osteoclasts in cultures of unfractionated bone cells. Unfractionated bone cells (4×10^5 cells/dentine slice in A and 10^7 cells/60-mm dish in B) were seeded, pretreated or not with Z-Asp-CH₂-DCB ($40 \mu\text{M}$) for 30 min, and then incubated with or without Dex (10^{-7} M) in the absence or presence of the inhibitor for 24 h. At the end of the culture, the pit area (solid bars in 'A'), the number of TRAP-positive MNCs (open bars in 'A') and the percentage of apoptotic MNCs (B) were measured. The experiments were performed three times, and the values shown are means $\pm$ SD for 5 cultures in a representative experiment. * $p < 0.01$ vs. untreated cells. NS, not significant.

factors (Suda et al., 1999). OCIF suppresses the survival and induces the apoptosis of osteoclasts (Akatsu et al., 1998; Takai et al., 1998). Murakami et al. (1998) recently reported that Dex stimulated the level of OCIF mRNA expressed in mouse primary osteoblastic cells, though the effect was small. Therefore, OCIF may be a mediator of the Dex effect on osteoclasts. However, the opposite effect of Dex on OCIF expression was more recently demonstrated in cultures of human osteoblastic cells (Hofbauer et al., 1999). In addition, our previous study (Hakeda et al., 1998) indicated that OCIF did not induce the apoptosis of rabbit isolated osteoclasts presented here. Therefore, other mechanisms may exist besides OCIF for the Dex effect.

GC-induced osteoporosis represents the most prevalent form of secondary osteoporosis, which is caused by strongly reduced bone formation and increased bone resorption (Lukert and Kream, 1996). Therefore, the data presented here seem to be at conflict with *in vivo* studies. The mechanism for the stimulatory effect of GCs on osteoclastic bone resorption remains unclear so far. The GC-induced increase in bone resorption was reported to be secondarily caused by increased parathyroid hormone (Adams et al., 1981; Bikle et al., 1993). However, administra-

tion of GCs to rats induced the inhibition of bone resorption in an early stage within one week, and thereafter, in contrast, greatly enhanced the resorption, whereas the bone formation decreased throughout this administration period (Koyama et al., 1998). Of the above diverse effects of GCs on bone resorption, the early response is consistent with our results indicating an inhibitory effect on mature osteoclasts, perhaps through non-osteoclastic cells. The later effect, enhancement of the bone resorption, may reflect a stimulatory effect on osteoclast generation. Osteoclastogenesis is up-regulated by many systemic hormones such as parathyroid hormone and 1,25-dihydroxyvitamin D₃ and local cytokines (Suda et al., 1996). GC-induced hyperparathyroidism and the subsequently-increased serum concentration of 1,25-dihydroxyvitamin D₃ may contribute to the enhancement of osteoclast formation. In addition, GCs has recently been demonstrated to stimulate the production of ODF/OPGL/TRANCE/RANKL, a strong and essential stimulator of osteoclastogenesis, by osteoblastic cells, suggesting a potent paracrine mechanism for GC-induced osteoporosis (Hofbauer et al., 1999). Taken together, the available data suggest that in a prolonged administration of GCs, the stimulatory effect of GCs on osteoclast development may overcome

the inhibitory effect on mature osteoclasts by excessively increasing the number of osteoclasts. Thus, the GCs effects on bone resorption are caused by complex actions and depend on the systemic and the local circumstances.

In conclusion, this study indicates that the main target cells of GCs are non-osteoclastic cells such as osteoblasts in bone and that GCs indirectly inhibits bone resorption by inducing apoptosis of the mature osteoclasts via the action of non-osteoclastic cells. This study expands our knowledge about the multifunctional roles of GCs in bone metabolism.

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