Role of Zinc in Regulation of Osteoclastogenesis

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Abstract
Zinc administration prevents bone loss in rats with ovariectomy, skeletal unloading, hydrocortisone treatment, adjuvant arthritics and diabetic condition in rats. Zinc has been shown to play a role in the preservation of bone mass by stimulating osteoblastic bone formation and inhibiting osteoclastic bone resorption in rat bone tissues. The cellular mechanism of zinc action in inhibiting osteoclastic bone resorption is reviewed. Zinc inhibits bone resorption which is stimulated by various bone-resorbing factors including parathyroid hormone (PTH), 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], and prostaglandin E₂ (PGE₂) in rat bone tissue culture in vitro. Also, zinc has an inhibitory effect on the bone-resorbing factors-induced osteoclast-like cell formation in mouse marrow culture in vitro. The stimulatory effect of bone-resorbing factors on osteoclastogenesis is mediated through receptor activator of NF-κB ligand (RANKL), which plays a key role in development of osteoclasts from preosteoclasts. Zinc inhibits RANKL stimulation in osteoclastogenesis. RANKL is expressed in osteoblasts. In addition, zinc may inhibit RANK expression which is mediated through Ca²⁺ signaling (protein kinase C) by stimulation of PTH, 1,25(OH)₂D₃ or PGE₂ in osteoblasts. Zinc plays an inhibitory role in the regulation of osteoclastogenesis.

Key words: zinc, osteoclastogenesis, bone resorption, osteoporosis

Introduction
Osteoporosis is widely recognized as a major problem of public health. The most dramatic expression of this disease is represented by fractures of the proximal femur for which the number increases as the population age [1, 2]. It is important to prevent bone loss with increasing age. The nutritional and pharmacological factors may have a role in preventing bone loss with aging [3]. This, however, is poorly understood.

Zinc has been demonstrated to have a wide variety of roles in the mammalian system, and this metal is essential for growth in human and many animals [4, 5]. Bone growth ratradation is a common finding in various conditions associated with zinc deficiency [6]. The cellular mechanism of zinc action has been demonstrated to stimulate proliferation and differentiation in osteoblastic cells [7-9]. Zinc can stimulate protein synthesis in osteoblastic cells [10]. Also, zinc has been shown to inhibit the formation of osteoclastic cells from bone marrow cells [11, 12], indicating that the metal has an inhibitory effect on bone resorption. Thus, zinc may play a role in the preservation of bone mass by stimulating bone formation and inhibiting bone resorption [12-14]. Zinc compound may be a good tool in therapy of osteoporosis. β-Alanyl-L-histidinato zinc [12, 13] and zinc acexamate [15] have a potent stimulatory effect on bone formation. Zinc compounds have a preventive effect on the decrease in bone mass in rats with skeletal unloading [16], feeding low-calcium and vitamin D-deficiency diet [17], hydrocortisone treatment [18], adjuvant arthritis [19], ovarian hormone deficiency [20] and diabetic condition [21]. The preventive effect of zinc compound on bone loss may be related to the stimulation of bone for-
mation and the inhibition of bone resorption, and as the result bone loss may be prevented.

Zinc has a physiologic role in the regulation of bone metabolism, and the metal compound has a therapeutic effect on bone disorder. However, the cellular and molecular mechanisms by which zinc regulates bone metabolism remain to be elucidated.

In this review, the author describes finding in regard to zinc action in osteoclast-like cell formation, which induces bone resorption, from marrow cell in vitro.

**Inhibitory Action of Zinc on Bone Resorption in Tissue Culture**

Zinc has been shown to inhibit various bone-resorbing factors-induced bone resorption in tissue culture system using rat calvaria in vitro [22]. Calvaria were removed from weanling rats (3-week-old male) and cultured for periods up to 48 hr in Dulbecco’s modified Eagle medium (high glucose, 4.5 %) supplemented with antibiotics and bovine serum albumin. The experimental cultures contained zinc compound. The bone resorbing factors, parathyroid hormone (1-34) (PTH; 10^{-8} M), prostaglandin E_2 (PGE_2; 10^{-5} M), interleukin-1 alpha (IL1_α; 50 U/ml), and lipopolysaccharide (LPS; 10 μg/ml), caused a significant decrease in bone calcium content. The decrease in bone calcium content induced by bone resorbing factors were completely inhibited by the existence of zinc compound (10^{-6} - 10^{-4} M).

Bone resorption stimulates production of lactic acid from osteoclasts in bone tissues [23]. Zinc compound (10^{-5} M) completely inhibited the PTH (10^{-7} M)- or IL1_α (50 U/ml)-induced increase in medium glucose consumption and lactic production by bone tissue, suggesting that the effects of zinc are partly related to the inhibition of acid production [22]. Furthermore, zinc compound (10^{-5} M) blocked both PTH (10^{-7} M)-induced acid phosphatase and decreased alkaline phosphatase activities of bone tissue [22]. Bone resorption is due to an increase in bone-resorbing activity of osteoclasts, which are mediated through PTH, PGE_2, IL1_α, and LPS [24].

**Inhibitory Effect of Zinc on Osteoclast-like Cell Formation in Bone Marrow Culture**

It is well established that osteoclastic cells are differentiated from marrow cells [25, 26]. Many bone resorbing factors can stimulate the formation of osteoclasts from marrow cells [27, 28]. Zinc has been demonstrated to have an inhibitory effect on osteoclastic-like cell formation in mouse culture in vitro [29]. The bone marrow cells were cultured for 7 days in α-minimal essential medium containing a well-known bone resorbing agent [1,25-dihydroxyvitamin D_3, PTH (1-34), IL1_α, and PGE_2]. Osteoclast-like cell formation was estimated by staining for tartrate-resistant acid phosphatase (TRACP), a marker enzyme of osteoclasts. The presence of 1,25-dihydroxyvitamin D_3{[1,25(OH)_2D_3; 10^{-6} M], PTH (10^{-6} M), IL1_α (50 U/ml) or PGE_2 (10^{-6} M) induced a remarkable increase in osteoclast-like multinucleated cells (MNCs). These increases were inhibited in the presence of zinc in the concentration range of 10^{-4}-10^{-5} M [29]. Zinc did not have an inhibitory effect on the proliferation of marrow cells, indicating that the metal do not exhibit cytotoxicity toward marrow cells.

The presence of Ni^{2+}, Cu^{2+}, or Co^{2+} (10^{-7} and 10^{-6} M) did not have an effect on PTH (10^{-7} M)-induced osteoclast-like cell formation. Thus, zinc had a potent inhibitory effect on osteoclast-like cell formation in mouse marrow culture [29]. Moreover, the inhibitory effect of zinc was equal in comparison with the effect of other anti-bone resorbing agents (calcitonin, 17β-estradiol, and acetazolamide) on osteoclast-like cell formation in mouse marrow culture [29].

**Characterization of Zinc Action in Osteoclast-like Cell Formation**

Zinc has been shown to stimulate the production of transforming growth factor-β (TGF-β) in osteoclastic MC3T3-E1 cells in vitro [30]. TGF-β plays a role as a coupling factor in bone formation and bone resorption [31, 32]. The growth factor has a regulatory effect on osteoclastic cell formation in vitro [33]. Whether zinc can modulate the effect of TGF-β on osteoclast-like cell formation in mouse marrow culture is examined. TGF-β has a stimulatory and an inhibitory effect on osteoclast-like cell formation in mouse marrow culture, and zinc has been demonstrated to inhibit the stimulatory effect of TGF-β [34]. This finding suggests that the effect of zinc in inhibiting osteoclast-like cell formation in mouse marrow culture is partly mediated through TGF-β, and that the metal inhibits the stimulatory effect of TGF-β in osteoclastogenesis.

It has been reported that bone resorbing factor [1,25(OH)_2D_3]-induced osteoclast-like cell formation in bone marrow cells is markedly enhanced by dexamethasone [35]. The steroid directly affects bone marrow cells and enhances osteoclast generation by inhibiting the endogenous production of granulocyte-macrophage colony stimulating factor (GM-CSF), which may function
as a negative regulation of osteoclast formation [35]. We showed that dexamethasone stimulates differentiation of bone marrow cells to preosteoclasts at an early stage of culture, and that this differentiation is not inhibited by zinc, supporting the view that zinc can inhibit differentiation from preosteoclasts to osteoclasts in bone marrow culture with dexamethasone [36]. Also, it is suggested that the inhibitory effect of zinc on osteoclasts-like cell formation does not result from the production of GM-CSF in marrow culture.

**Involvement of Intracellular Signaling Factor of Zinc Action in Osteoclast-like Cell Formation**

The cellular mechanism of zinc action inhibiting the PTH-induced osteoclast-like cell formation in mouse marrow culture *in vitro* has been shown [37,38]. PTH-stimulated osteoclast-like cell formation from hemopoietic blast cells linked to the activation of cyclic AMP-dependent protein kinase and protein kinase C [39]. The effect of PTH is mediated through two second messenger signalings of cyclic AMP and Ca\(^{2+}\).

The effect of zinc in inhibiting the PTH-induced osteoclast-like cell formation was seen in the absence or presence of theophylline, which can inhibit the activity of cyclic AMP phosphodiesterase [37]. However, zinc did not inhibit the stimulatory effect of dibutyryl cyclic AMP on osteoclast-like cell formation. The stimulatory effect of PTH on osteoclast-like cell formation was clearly weakened in the presence of EGTA or dibucaine. Phorbol 12-myristate 13-acetate (PMA), a protein kinase C activator, clearly stimulated osteoclast-like cell formation. The PMA effect was inhibited in the presence of zinc. However, the inhibitory effect of zinc was not seen in the presence of both PTH and PMA. From these findings, it is assumed that zinc does not influence on cyclic AMP-dependent mechanism in the stimulation of osteoclast-like cell formation by PTH. Zinc may act on the process of PTH-induced activation of protein kinase C which is involved in Ca\(^{2+}\)-signaling in marrow cells [38]. It has been demonstrated that four atoms of zinc bind tightly to the regulatory domain of protein kinase C, which appears to be a requirement for the binding of phorbol ester to this region [40]. Zinc may influence on protein kinase C activity which is involved in Ca\(^{2+}\)-signaling of osteoclastogenesis.

It is well established that receptor activator of NF-κB ligand (RANKL) plays a key role in development of osteoclasts from preosteoclasts [25,26]. RANKL is secreted from osteoblasts. PTH, 1,25(OH)\(_2\)D\(_3\), and PGE\(_2\), which are bone-resorbing factors, stimulates the expression of RANKL in osteoblasts. RANKL binds to RANK (receptor for RANKL) in preosteoclasts, and stimulates differentiation to osteoclasts. The expression of RANKL in osteoblasts is mediated through Ca\(^{2+}\)signaling which is involved in protein kinase C in osteoblasts, and that RANKL expression is suppressed. The decrease in RANKL may result in suppression of osteoclastogenesis.

**Action of Zinc on RANKL-stimulated Osteoclastogenesis**

RANKL is a member of the tumor necrosis factor (TNF) superfamily, which was originally identified as a T-cell-derived immunomodulatory cytokine [41]. RANKL is expressed in activated T cells and promotes the survival of dendritic cells by binding to its receptor RANK, which results in the enhanced dendritic cell-mediated T cell proliferation. RANKL/RANK pathway is essential for osteoclast differentiation [25, 26]. RANKL expression is induced in osteoblastic cells and bone marrow stromal cells in response to osteotropic factors such as 1,25(OH)\(_2\)D\(_3\), PTH, and PGE\(_2\), and combined treatment of hematopoietic cells with macrophage-colony stimulating factor (M-CSF) and the soluble from of RANKL (sRANKL) induced osteoclast differentiation *in vitro* [42]. The effect of RANKL was completely abrogated by adding a natural antagonist of RANKL, osteoprotegerin (OPG) [43]. TNF receptor-associated factor (TRAF) family proteins are adaptor molecules that mediate intracellular signaling of various cytokine receptors including the TNF receptor superfamily and Toll/interleukin receptor (IL-1R) family [44]. TRAF6 has the most divergent TRAF-C domain, and it is the only TRAF that is involved in the signal from members of the Toll/IL-1R family by interacting with the IL-1R-associated kinase (IRAK). TRAF2, TRAF3, and TRAF5 bind to the membrane-distal domain in the cytoplasmic fail of RANK, whereas TRAF6 interacts with the membrane-proximal domain. TRAF6 binds to the membrane-proximal region of RANK and IL-1R-associated kinase (IRAK), and is critically involved in the intracellular signal transduction including NF-κB and nitrogen-activated protein kinase (MAPK) activation.

Zinc has been shown to have an inhibitory effect on RANKL-induced osteoclast-like cell formation in mouse marrow culture in the presence of M-CSF [45]. Also, zinc inhibited TNFα-induced osteoclastogenesis [45]. TNFα is an autocrine factor in osteoclasts, promoting their differentiation, and mediates, at least in part, RANKL's
induction of osteoclastogenesis [46]. The inhibitory effect of zinc on osteoclastogenesis may be partly involved in the suppressive effect on RANKL stimulation (Fig.1). In addition, zinc may inhibit signaling pathway which is related to RANKL stimulation in preosteoclasts.

The effect of zinc in inhibiting RANKL-induced osteoclastogenesis was found to be abolished in the presence of cycloheximide, an inhibitor of protein synthesis, or 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), an inhibitor of transcription in mouse marrow culture [45]. This finding suggests that the inhibitory effect of zinc on osteoclastogenesis is partly resulted from a newly synthesized protein component in mouse marrow culture. It is speculated that zinc stimulates the expression of RANKL inhibitor (including OPG) in osteoblasts, and that the metal induces a factor which can suppress osteoclast development in preosteoclasts (Fig. 1).

Conclusion

Zinc is essential for growth in human and many animals, and it stimulates bone growth. Zinc plays a physiologic role in the preservation of bone mass by stimulating bone formation and inhibiting bone resorption. The activation of osteoclast induces bone resorption. Zinc has been shown to inhibit osteoclastic bone resorption induced by bone-resorbing factors including PTH, 1,25(OH)₂D₃, PGE₂, lipopolysacharide, and TNF-α. These factors are related to RANKL/RANK system which stimulates osteoclastic development. RANKL is expressed in osteoclast, and its expression is stimulated by PTH, 1,25(OH)₂D₃, PGE₂, and TNF-α. RANKL plays a pivotal role in osteoclast development. Zinc has been demonstrated to suppress RANKL stimulation in osteoclast development. The inhibitory action of zinc on osteoclastogenesis may be resulted from the suppression of RANKL stimulation in preosteoclasts. Moreover, zinc may inhibit RANK expression which is mediated through Ca²⁺ signaling (protein kinase C) by stimulation of bone-resorbing factors in osteoblasts. Zinc plays a regulatory role in osteoclastogenesis.

References

7) Hashizume M and Yamaguchi M: Stimulatory effect
of $\beta$-alany-L-histidinate zinc on cell proliferation is dependent on protein synthesis in osteoblastic MC3T3-E1 cells. Mol Cell Biochem 122: 59-64, 1993.


33) Shimar DM and Rodan GA: Biphasic effects of transforming growth factor-α on the production of osteoclast-like cells in mouse bone marrow cultures: The role of prostaglandins in the generation of these cells. Endocrinology 126:3153-3158, 1990.


