KOREA J. ORTHOD. 2000 : 30(6) : 713-21

Interleukin-1β induces bone resorption by regulation of prostaglandin E_2 synthesis and plasminogen activator activity, and TGF-β inhibits bone resorption of rat bone cells

Young-Hun Kim[1] · Young-Jun Lee[2] · Kyu-Rhim Chung[3] · Young-Guk Park[4]

Bone cells produce multiple growth factors and cytokines that have effects on bone metabolism and can be incorporated into the bone matrix. The present study was designed to extend these observations by examining the interactions between transforming growth factor-β (TGF-β) or interleukin-1β (rhIL-1β) and bone cells in a rat long bone culture model. IL-1β regulates several activities of the osteoblast cells derived from rat long bone explants in vitro. IL-1β stimulated cellular proliferation as well as the synthesis of prostaglandin E_2 and plasminogen activator activity in the cultured cells in a dose-dependent manner. TGF-β is present in the bone matrix and potentially released during bone resorption. TGF-β reduced basal bone resorption and inhibited vitamin D_3 [1,25$(OH)_2D_3$]-induced bone resorption in rat long bone cells. These results support the role of IL-1β in the pathological modulation of bone cell metabolism, with regard to implication in the pathogenesis of osteoporosis by IL-1β, and that TGF-β positively inhibits the bone resorption.

Key words : Interleukin 1-β, Tranforming Growth Factor-β, osteoblast, osteoclast, Prostaglandin E_2, Plasminogen activator, bone resorption

Bone cells have been shown to produce a wide array of proteins, including growth factors, which have effects on bone metabolism and can be released into bone matrix. Among these are members of transforming growth factor-β (TGF-β) superfamily, which affect osteoblast cell replication and differentiation[1]. TGF-β is a stimulator of bone formation, which is locally produced by osteoblasts[2] and osteoclasts[3] and is secreted and stored in an inactive form in bone[4,5]. TGF-β can be released from the extracellular matrix during bone resorption by osteoclasts[3] and it modulates the expression of several markers of the osteoblast phenotype [6-10].

A number of factors functions as regulators of proliferation, differentiation, and maintainance of the diverse functions of osteoblasts and osteoclasts. The resident immune cells and their products are potentially contributed to the localized remodeling processes involved in bone metabolism[11]. Studies have demonstrated that one such cytokine, interleukin-1 (IL-1), modulates several aspects of the activity of various bone cell types. IL-1 is a potent stimulator of bone resorption in vitro[12], and manifests an action partially mediated via the stimulation of osteoclast cell formation indicating the modulation of osteoblast cell

[1] Graduate Student, Kyung Hee University
[2] Assistant Professor of Orthodontics, Kyung Hee University
[3] [4] Professor of Orthodontics, Kyung Hee University

Reprint requests to : Prof. Young-Guk Park
Hoeki-dong 1, Dongdaemoon-ku, Seoul 130-701, Korea
Department of Orthodontics, Kyung Hee University
82-2-958-9395 / ygpark@khu.ac.kr

activity by IL-1 [13]. Previous studies have demonstrated that IL-1β regulates several aspects of the functional activity of human osteoblast-like cells in vitro[13,14].

It is also a potent inducer of prostanoids[15,16]. Both prostaglandin (PG)-dependent and PG-independent effects on bone metabolism have been reported. For example, injection of IL-1 over the calvariae of mice caused a short-term PG-independent stimulation of bone resorption followed by a prolonged increase in resorption, which was PG-dependent[17]. The production of PGE_2 is closely regulated by cytokines such as IL-1 and tumor necrosis factor-α[15]. It has also been shown to be a potent stimulator of prostaglandin G/H synthase-2 expression in murine osteoblastic cells[18]. The enzyme is generally expressed at low levels in most tissues but can be rapidly and transiently induced to high levels by multiple factors, including cytokines, growth factors, and tumor promoters[19]. Many of the important regulators of bone metabolism including cytokines, such as IL-1[20], IL-6 [21], and tumor necrosis factor-α[22], growth factors, such as transforming growth factor-α[20], transforming growth factor-β[20,23], and basic fibroblast growth factor [24], hormons, such as parathyroid hormone[25] and cortisol[18,23], PGs themselves[18,23], and mechanical forces[26] have been shown to regulate PG production in rodent osteoblastic cells[27].

It is the aim of the study to examine whether these effects are exhibited by recombinant human IL-1β (rhIL-1β) on rat long bonel osteoblast cells derived from fetal rat, and to extend the observations on action of transforming growth factor-β in osteoblasts as well as bone resorption in a long bone culture model.

MATERIALS AND METHODS

Materials

1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$; Vit D] was purchased from Sigma Co. LTD. (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), bovine serum albumin, cycloheximide (CHM), indomethacin and other chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA). PGE_2 antibody was purchased from Immunoassay Co. (Tokyo, Japan). [^{3}H]-Prostaglandin was purchased from New England Nuclear (NEN, Boston, MA.). TGF-β type 2 was purchased from Immunex, Inc. (Seattle, WA.). ^{45}Ca (NEN, Boston, MA) was purchased from supplier. All other reagents were of the best grade commercially available.

Rat bone cell culture

Explants of rat long bone were cultured as described[13]. The cells obtained have been routinely characterized and shown to express an osteoblast-like phenotype in culture. Recombinant pure human IL-1β (specific activity 5 x 10^5 U/mg) was our deposit[28], which was a gift of Dr. Park SH, Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea Institute of Science and Technology(KIST).

Cell proliferation

Cell proliferation was assessed by the incorporation of [^{14}C]-thymidine into materials precipitable by trichloroacetic acid. Cells were pulsed for the final 24 h of a 46 h incubation period.

Alkaline phosphatase assay

Alkaline phosphatase activity in the solubilized cell layer was measured by monitoring the release of p-nitrophenol from disodium p-nitrophenyl phosphate. The assay buffer consisted of 0.1 M diethanolamine, supplemented with 0.5 M magnesium chloride (pH 10.5). Results are expressed as μmoles per μg cell protein per h.

PG assay

PGE_2 released into the culture medium over 72 h incubation period was measured by radioimmunoassay using an antiserum with specificity towards PGE_2 (Immunoassay, Co., Tokyo Japan) as described[11]. Results are expressed as ng PGE_2 per μg cell protein.

Bone resorption assay

The effects of systemic and local factors on bone resorption were assessed by measuring the percentage of ^{45}Ca release from prelabeld 17-day-old fetal rat radii and ulnae after 3 and 6 days of culture. The fetal rat long bone organ tissue culture system was based on that described by Raisz[29]. Fetal bones were labeled with ^{45}Ca by injecting the mother with 200 μCi ^{45}Ca (NEN, Boston, MA) on the eighteenth day of gestation. Radii and ulnae bone shafts were obtained from 19 day fetuses by microdissection. The shafts were cut beyond the calcified zone and therefore contained short lengths of cartilage at the ends. Bones were cultured on sunk Millipore filter dots in 24-well Limbro plates. The shafts were first cultured in 0.5 ml BGJ_b medium (Gibco Laboratories, Grans Islan, NY) containing 1.0 ml/ml bovine serum albumin, 100 units/ml penicillin G, and 1 μg/ml polymyxin B for 1 day to reduce exchangeable ^{45}Ca. One bone from a fair (right and left radii or right and left ulnae from a single fetus) was then transferred into medium containing agonist(s) (treatment) and the contralateral bone was placed into identical medium without agonist(s) (control). A typical test group consisted of 5 pairs of bones. Bones were cultured for 5 days in a 95% air/5% CO_2 incubator at 37℃ and 95% humidity with one change of media after 2 days. The percentage of ^{45}Ca released from a bone into the medium during the 5-day culture was determined by measuring the radioactivity in medium 1, medium 2, and the trichloroacetic acid solubilized bone using a liquid scintillation counter. Stimulated resorption was expressed as the paired difference between treatment and control bone percent ^{45}Ca released from during the 5-day culture. Dead bone ^{45}Ca release in this system was approximately 10%. BGJ_b control ^{45}Ca release was 16-20% and maximum IL-1b ^{45}Ca release was 60-80%. Since "stimulated" release is expressed as the mean difference between paired BGJ_b control bones (C%) and treated bones (T%), the T%-C% for an inactive treatment is zero, and a maximum IL-1β response is approximately 40-60%. Each bone was labeled with approximately 20,000 CPM ^{45}Ca. In specific case, $1,25(OH)_2D_3$ as a bone resorption agent was used.

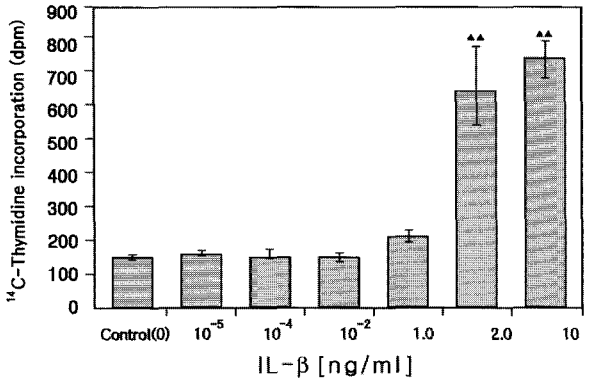

Fig. 1. Effects on cell growth of the rat bone osteoblasts by IL-1β. Cell growth was assessed by [^{14}C]-thymidine incorporation. Values represent mean ± S.E.M (n=5). Significant difference from control, *P<0.05, ** P<0.005.

Statistics

Statistical differences between treatments were determined using analysis of variance. All other statistical anaylses were done by Student's t-test.

RESULTS

Effects on cell proliferation, PGE_2 production and plasminogen activator activity of the rat osteoblasts by IL-1β

The proliferation of the rat osteoblasts was stimulated in a dose-dependent manner by IL-1β over the concentration range of 0.01 ng - 2 ng/ml (Fig. 1). The stimulation of cell proliferation came to peak level at 2.0 ng/ml, while concentrations below 0.1 ng/ml had no detectable effect. Correspondingly, IL-1β stimulated the production of PGE_2 in a dose-dependent manner over the concentration range of 0.01 ng - 2 ng/ml with a maximal effect being observed at 2 ng/ml (Fig. 2). The stimulation of cell proliferation was most pronounced at 2.0 ng/ml, while concentrations below 1.0 ng/ml exhibited no detectable activity on the synthesis of PGE_2.

The plasminogen activator activity of the rat oste

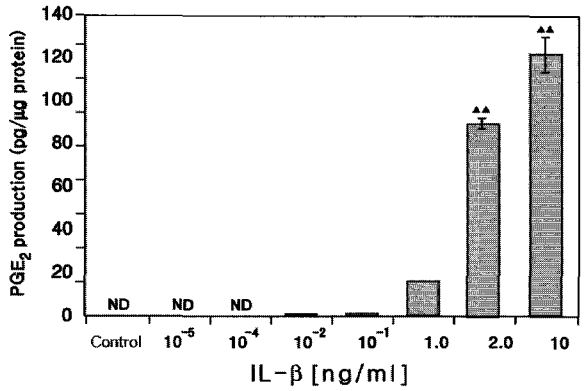

Fig. 2. Effects of IL-1β on PGE_2 prodcution by rat bone cells PGE_2 released into the culture media was measured. Values represent mean ± S.E.M (n=5). Significant difference from control. *P<0.05, ** P<0.005.

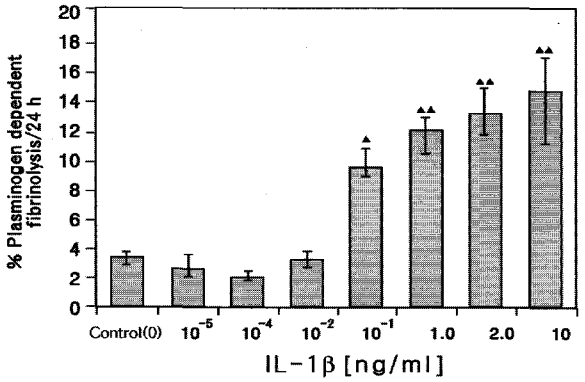

Fig. 3. Stimulation of the plasminogen activator activity of rat bone cells including osteoblasts and osteoclasts by IL-1β. Plasminogen activator activity of rat osteoblasts induced by rhIL-1β was measured. The data shown represents the plasminogen dependent fibrinolysis of [^{125}I] -fibrin substrate. Values represent mean ± S.E.M (n=5). Significant difference from control.
*P<0.05, ** P<0.001.

oblast was likewise stimulated by IL-1β in dose-dependent manner over the dosage range of 0.01 ng -2 ng/ml with a utmost effect being observed at 2 ng/ml (Fig. 3). The plasminogen activator activity was significantly stimulated compared to that control. Concentrations below 0.1 ng/ml exhibited no detectable effect on the plasminogen activator activity.

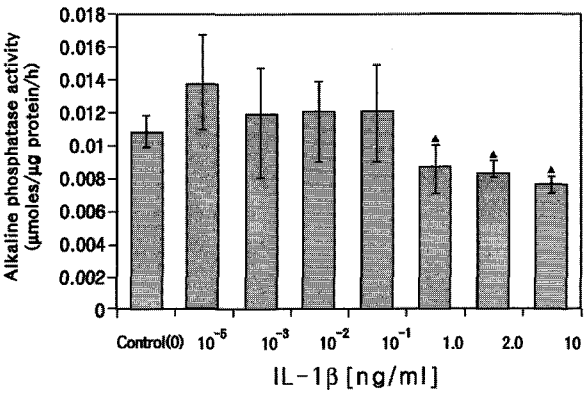

Fig. 4. Effect of IL-1β on alkaline phosphatase activity in the rat osteoblastic cells. The alkaline phosphatase activity of the solubilized cell layer of the rat osteoblastic cells was measured. Values represent mean ± S.E.M (n=5). Significant difference from control. *P<0.05, ** P<0.001.

Effect of IL-1β on alkaline phosphatase activity in the rat long bone cells

To examine the effects of IL-1β on alkaline phosphatase activity in the rat long bone cells, various concentrations IL-1β were treated to the cells and alkaline phosphatase activities were assayed. The basal alkaline phosphatase activity of the rat osteoblast cells was decreased by IL-1β over the dose range of 0.01 -2.0 ng/ml (Fig. 4).

Stimulation of IL-1β on bone resorption

Treatment of rat long bone cells with IL-1β resulted in dose dependent stimulation of bone resorption. The dose response for stimulating bone resorption differed significantly between the fetal rat long bone organ tissue culture (unpublished data) and this culture system of rat long bone cells. As shown in Fig. 5, human IL-1β has a potential in stimulating bone resorption as measured by means of calcium release when each is normalized to nano gram of amounts.

Effect of TGF-β on 1,25$(OH)_2D_3$ (Vit D)-induced bone resorption

A conspicuous effect of 1,25$(OH)_2D_3$ is stimulation of

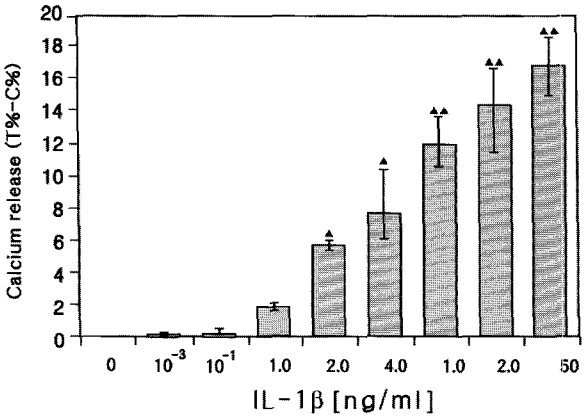

Fig. 5. Dose-dependent bone resorption of IL-1β in rat bone cells. Bone resorption was measured as percent release of ^{45}Ca during 5 days of rat long bone cell culture. Each point is the mean paired difference ± S.E. for 5 treatment-control bone pairs. ** P<0.001.

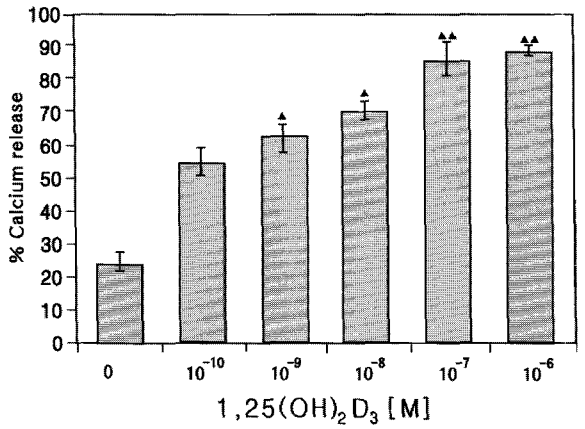

Fig. 6. The effect of $1,25(OH)_2D_3$ on bone resorption in fetal rat long bone cells. Prelabeled fetal rat radii /ulnae were incubated with the indicated concentrations of $1,25(OH)_2D_3$ for 5 days, and the release of ^{45}Ca into the medium was determined. Percent release of ^{45}Ca is a measure for bone resorption and was assessed as described. Each point is the mean paired difference ± S.E. for 5 cultures. * P<0.05: ** P<0.001.

bone resorption. As shown in Fig. 6, $1,25(OH)_2D_3$ dose dependently stimulated bone resorption in the fetal rat radii/ulnae as measured by release of prelabeled ^{45}Ca. This effect was observed after 3 days and most pronounced after 6 days of incubation.

Incubation of fetal rat long bones with TGF-β caused small yet significant inhibition of basal bone

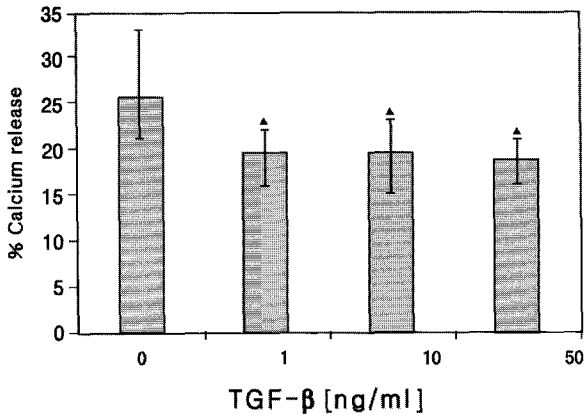

Fig. 7. The effect of TGF-β on non-stimulated bone resorption in fetal rat long bone cells. Prelabeled fetal rat radii/ulnae were incubated with the indicated concentrations of TGF-β (1-1000 ng/ml) in the absence of $1,25(OH)_2D_3$ for 5days (two times 3 days). Data obtained after 5 days are presented. After 3 days, similar but less-distinguished effects were observed. Data are presented as ± SEM of 5 cultures. * P<0.05: ** P<0.001.

resorption after 6 days (Fig. 7). As shown in Fig. 7, after 6 days of culture, the incubation of bone resorption by $1,25(OH)_2D_3$ (Vit D, 10^{-7} M) was inhibited in dose-responsive manner by 10 and 50 ng/ml TGF-β (Fig. 8). The effect of TGF-β after 3 days of incubation was analogous but less distinguishable as compared with the effects after 6 days of incubation.

DISCUSSION

In bone resorption reaction, it has been known that IL-1β is responsible to progessive degradation of bone by activating osteoblast cells and by causing the progenitor cells to mature cells[11]. IL-1β stimulated the plasminogen activator activity of the rat osteblast cells in a dose-dependent manner. The stimulation of plasminogen activator activity by IL-1β has been observed in several connective tissue cell types, including human osteoblast-like cells[13]. IL-1β is a potent stimulator of bone resorption both in vitro[11] and in vivo[12] through an action which may be mediated primarily via the osteoblast[12,13]. The observation that IL-1β stimulated the plasminogen activator activity of the rat osteoblast cells may indicate a potential mechanism for the osteoblast-mediation of bone resorption. Plasmino-

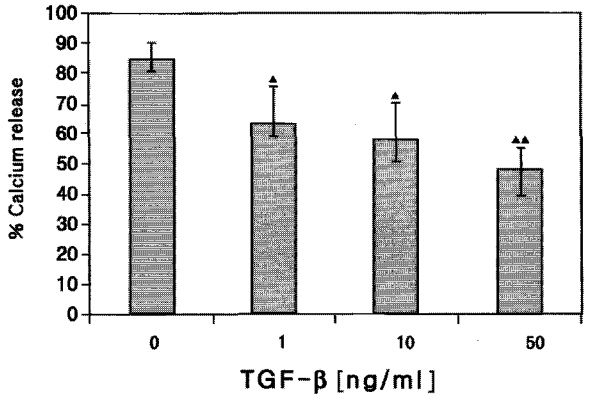

Fig. 8. The effect of TGF-β on 1,25$(OH)_2D_3$-stimulated bone resorption in fetal rat long bone cells. Prelabeled fetal rat radii/ulnae were incubated with the indicated concentrations of TGF-β (1- 1000 ng/ml) in the presence of 10^{-7} M 1,25$(OH)_2D_3$ for 5 days (two times 3 days). Data obtained after 5 days are presented. After 3 days, similar but less- distinguished effects were observed. Data presented as ± SEM of 5 cultures. * P<0.05: ** P<0.001.

gen activator has been associated in several processes governing connective tissue degradation. The existence of a plasmin-dependent proteolytic system in mineralized matericas has been known and so may contribute to the breakdown of the constitutents of the bone matrix[13]. One mechanism whereby this may be achieved is the activation of latent collagenase via action of plasmin, following its generation from plasminogen[11,13]. Rodent osteoblast cells produce latent collagenase in response to several bone resorbing a of PGE_2 production by IL-1β and resulting stimulation of bone resorption can occur partially via PGE_2- dependent mechanism, signifying that the PGE_2 synthesis by osteoblast-like cells in response to IL-1β may contribute to this effect. Elevated production of IL-1β has been implicated in the pathogenesis od osteoporosis and with humoral hypercalcemia associated with squamous cell carcinomas. The present study therefore provides further support for the role of IL-1β in the bone metabolism process. In addition, the demonstration of the production of IL-1β-like factors by osteoblasts may indicate a potential paracrine/ autocrine regulatory mechanism affecting both osteoblasts and osteoclasts and consequently the cellular processes governing the regulation of bone metabolism. The capability of IL-1β to suppress these markers of the mature osteoblast in association with the stimulation of cell proliferation is intriguing. Elevated production of IL-1β has been implicated in the pathogenesis of osteoporosis and with humoral hypercalcemia associated with some carcinomas. Thus, the present study provides further support for the role of IL-1β in the processes governing bone metabolism.

On the other hand, the present results support the hypothesis that bone-derived growth factors interact with the systematic regulator of bone metabolism, i.e., 1,25$(OH)_2D_3$, in the control of bone turnover as assessed by the regulation of bone resorption. Previously, Staal et al. demonstrated that TGF-β potently blocks 1,25$(OH)_2D_3$-stimulated osteocalcin expression in fetal rat and human osteoblastic cells via interference at the transcriptional level[31,32]. They found that in fetal mouse long bones, 1,25$(OH)_2D_3$ dose-dependently reduced osteocalcin synthesis[9].

TGF-β reduced the basal osteocalcin level in the fetal rat long bone cultures (data not shown). This inhibition is in line with observations with rat and human osteoblast-like cells[33,34,35]. This growth factor is produced by bone cells and incorporated into the bone matrix[9]. This implicates that this factor defines the action of systematic factors at specific sites and may be substantial in the regulation of bone formation. For instance, 1,25$(OH)_2D_3$-induced bone resorption at TGF-β-positive site may be limited whereas the resorption can go on at TGF-β-negative site. This model requires the release of this factor as a biologically active form from the bone matrix during resorption. TGF-β is existed in a latent form in the bone matrix[34]. The present data on the inhibitory effect of TGF-β on 1,25$(OH)_2D_3$-stimulation of bone resorption appears to be protective effects for bone formation as TGF-β is a potent inhibitor of 1,25 $(OH)_2D_3$-induced osteoblast-like cell formation in long-term human bone marrow cultures, both by inhibiting proliferation and differentiation of early precursors and by inhibiting fusion of mononuclear cells to form osteoclasts[35]. In addition, TGF-β inhibition of 1,25$(OH)_2D_3$-stimulated bone resorption was suggested to occur via the inhibition of osteoclast precursor proliferation in fetal rat long

bones[36].

In conclusion, the data on the effects of TGF-β on bone cells and its interaction with $1,25(OH)_2D_3$, systematic regulator of bone metabolism, at osteoclast level has led to the hypothesis that this plays an important role in bone metabolism. The present study supports this concept by growth factor-specific interaction between TGF-β and $1,25(OH)_2D_3$ with respect to bone resorption. Besides the present study provides a basis for a local regulatory mechanism of bone resorption stimulated by $1,25(OH)_2D_3$.

SUMMARY AND CONCLUSION

Many of the pertinent regulators of bone metabolism including cytokines, such as IL-1, IL-6, and tumor necrosis factor-α, growth factors, such as transforming growth factor-α, transforming growth factor-β, and basic fibroblast growth factor, hormones, such as parathyroid hormone and cortisol, PGs themselves, and mechanical forces have been shown to regulate PG production in rodent osteoblastic cells. This study was undertaken to scrutinize whether these effects are exhibited by recombinant human IL-1β (rhIL-1β) on rat long bone osteoblast cells derived from fetal rat, and to extend the observations on action of transforming growth factor-β in osteoblasts as well as bone resorption in a long bone culture model.

Bone cell culture from explants of rat long bone, cell proliferation assessment by the incorporation of [^{14}C]-thymidine, alkaline phosphatase assay by monitoring the release of p-nitrophenol from disodium p-nitrophenyl phosphate, PG assay by radioimmunoassay using an antiserum with specificity towards PGE_2, bone resorption assay by measuring the percentage of ^{45}Ca release from prelabeled bone were executed.

IL-1β stimulated the proliferation of osteoblasts in dose-dependent manner over the concentration of 0.01-2.0ng/ml, the production of PGE_2 in dose-dependent manner over 0.01-2.0ng/ml with a maximal effect at 2.0 ng/ml, and plaminogen activator activity. The basal alkaline phosphatase activity of osteoblasts manifested diminution by IL-1β over 0.01-2.0 ng/ml. Treatment of bone cells with IL-1β resulted in dose-dependent stimulation of bone resorption. $1,25(OH)_2D_3$ dose dependently stimulated bone resorption. Incubation with TGF-β caused minute, yet significant inhibition of bone resorption. The incubation of bone resorption by $1,25(OH)_2D_3$ was inhibited in 10-50 ng/ml TGF-β.

These consequences advocate the role of IL-1β and TGF-β in the orthodontic modulation of bone cell metabolism in paradental tissues.

REFERENCES

1. Canalis E, Pash J, Varghese S. Skeletal growth factors. Crit. Rev. Eukaryot Gene 1993 : 3 : 155-66.
2. Robey PG, Young MF, Flanders KC, et al. Osteoblasts synthesize and respond to transforming growth factor-β in vitro. J Cell Biol 1987 : 105 : 457-63.
3. Oursler MJ. Osteoclast synthesis and secretion and activation of latent transforming growth factor-β. J Bone Min Res 1994 : 9 : 443-52.
4. Sporn MB, Roberts AB, Wakefield LK, de Crombrugghe B. Somre Recent advances in the chemistry and biology of transforming growth factor-β. J Cell Biol 1987 : 105 : 1039-45.
5. Mssague J. The transforming growth factor-β family. Ann Rev Cell Biol 1990 : 6 : 597-641.
6. Rosen DM, Stempien SA, Thompson AY, Seyedin SM. Transforming growth factor-β modulates the expression of osteoblast and chondroblast phenotypes in vitro. J Cell Physiol 1988 : 134 : 337-46.
7. Wrana JL, Maeno M, Hawrylyshyn B, et al. Differential effects of transforming growth factor-β on the synthesis of extracelluar matrix proteins by normal fetal rat calvarial bone cell populations. J Cell Biol 1988 : 106 : 915-24.
8. Bonewald LF, Dallas SL. Role of active and latent transforming growth factor-β in bone formation. J Cell Ciochem 1994 : 55 : 350-7.
9. Staal A, Ceertsma-Kleinekoort WMC, Van Den Bemd GJCM, et al. Regulation of osteocalcin production and bone resorption by 1,25-dihydroxyvitamin D_3 in mouse long bones : Interaction with bone-derived growth factors TGF-β and IGF-I. J. Bone Min. Res. 1998 : 13 : 36-43.
10. Rodan GA, Noda M. Gene expression in osteoblastic cells. Crit. Rev. Eukaryot Gene Expr 1991 : 1 : 85-98.
11. Baron R, Vignery A. Horowitz, M. In Bone and Mineral Research. Elsevier, Amsterdam. 1983 : Annual 2 (W. A. Peck, ed) : 175-243.
12. Hazuda DJ, Strickler J, Hueppers F, Simon PL. Young, PR. Processing of precursor Interleukin 1β and inflammatory disease. PA. J. Biol. Chem. 1990 : 265 : 6318-22.
13. Evans DB, Bunning RAD. Russell RGG. The effects of recombinant human interleukin-1β on cellular proliferation and the production of prostaglandin E2, plasminogen activator, osteocalcin and alkaline phosphatase by osteoblast-like cells derived from human bone. Biochem. Biophys. Res. Comm. 1990 : 166 : 208-16.
14. Heasman PA, Collins JG, Offenbacher K. Changes in crevicular fluid levels of inteleukin-1β, leukotriene B_4, prostaglandin E_2, thromboxane B_2 and tumor necrosis factor α in experimental gingivities in humans. J Periodont Res. 1993 : 28 : 241-7.
15. Harrison JH, Lorenzo JA, Kawaguchi H, Raisz LG, Pilbeam CC. Stimulation of prostaglandin E2 production by interleukin-1 α and transforming growth factor α in osteoblastic MC3T3-E1 cells. J Bone Miner Res 1994 : 9 : 817-23.

16. Sato K, Fuji Y, Kasono K, et al. Stimulation of prostaglandin E2 and bone resorption by recombinant human interleukin-1 α in fetal mouse bone. Biochem Biophys Res Comm 1986 : 138 : 618-24.
17. Boyce BF, Aufdemorte TB, Garrett IR, Yates AJP Mundy GR. Effects of interleukin-1 on bone turnover in normal mice. Endocrinology 1989 : 125 : 1142-50.
18. Kawaguchi H, Raisz LG, Vosnesensky OS, et al. Regulation of the two prostaglandin G/H synthases by Parathyroid hormone, interleukin-1, cortisol and prostaglandin E2 in cultured neonatal mouse calvariae. Endocrinology 1994 : 135 : 1157-64.
19. Herschman HR. Regulation of prostaglandin synthase-1 and prostaglandin synthase-2. Cancer Metastasis Rev. 1994 : 13 : 241-56.
20. Pilbeam C, Rao Y, Voznesensky O, et al. Transforming growth factor β1 regulation of prostaglandin G/H synthase-2 expression in osteoblastic MC3T3-E1 cells. Endocrinology 1997 : 138 : 4672-82.
21. Tai H, Miyaura C, Pilbeam CC, et al. Transcriptional induction of cycloixygenase-2 in osteoblasts is involved in interleukin-6-induced osteoclast formation. Endocrinology 1997 : 138 : 2372-9.
22. Kawaguchi H, Nemoto K, Raisz LG, et al. Interleukin-4 inhibits prostaglandin G/H synthase-2 and cytosolic phospholipase A2 induction in neonatal mouse parietal bone cultures. J Bone Miner Res 1996 : 11 : 358-66.
23. Pilbeam C. Kawaguchi H, Hakeda Y, et al. L. Differential regulation of inducible and constitutive Prostaglandin endoperoxide synthase in osteoblastic MC3T3-E1 cells. J Biol Chem 1993 : 268 : 25643-9.
24. Kawaguchi H, Pilbeam C, Gronowicz G, et al. Transcriptional induction of prostaglandin G/H synthase-2 by basic fibroblast growth factor. J Clin Invest 1995 : 96 : 923-30.
25. Tetradis S, Pilbeam C, Liu Y, Kream ME. Parathyroid hormone induces prostaglandin G/H synthase-2 expression by a cyclic adenosine 5'-monophosphate-mediated pathway in the murine osteoblastic cell line MC3T3 -E1. Endocrinology 1996 : 137 : 5435-40.
26. Pilbeam C, Raisz L, Voznesensky O, et al. Autoregulation of inducible prostaglandin G/H synthase in osteoblastic cells by prostaglandins. J Bone Miner Res 1994 : 10 : 406-14.
27. Pilbeam C, Harrison JR. and Raisz LG. Prostaglandins and bone metabolism. In Bilezikian JP, Raisz LG, Rodan GA. (eds.) Principles of Bone Biology. CA San Diego, Academic Press 1996 : 715-28.
28. Park C, Kim JR, Shim JK, et al. Inhibitory effects og streptozotocin, tumor necrosis factor-α, and interleukin-1β on glucokinase activity in pancreatic islets and gene expression of GLUT2 and glucokinase Arch Bioche Biophys 1999 : 362 : 217-24.
29. Raisz LG. and Niemann I. Effect of phosphate, calcium and magnesium on bone resorption and hormonal responses in tissue culture. Endocrinology 1969 : 85 : 446-52.
30. Meikle MC, Bord S, RM Reynolds JJ. Rabbit calvarial osteoblasts in culture constitutively synthesize progelatinase-A, and TIMP-1 and TIMP-2. Biochim. Biophys. Acta 1994 : 1224 : 99-102.
31. Staal A, Bierkenhager HC, Pols HAP, et al. TGF-β -induced dissociation between vitamin D receptor level and, $1,25(OH)_2D_3$ action in osteoblast-like cells. Bone Miner 1994 : 26 : 27-42.
32. Staal A, van Wijnen AJ, Desai RK, et al. Antagonistic effects of TGF -β on $1,25(OH)_2D_3$-enahancement of osteocalcin and osteopontin transcription : reduced interactions of vitamin D receptor/retinoic X receptor complexes with Vitamin D response elements. Endocrinology 1996 : 137 : 2001-11.
33. Banerhee C, Stein JL, van Wijnen AJ, et al. TGF-β1 responsiveness of the rat osteocalcin gene is mediated by an activator protein-1 binding site. Endocrinology 1996 : 137 : 1991-2000.
34. Nonewald LF, Wakefield L, Oreffo RO, et al. Latent forms of TGF-β derived from bone cultures : Identification of a naturally occuring 100-kDa complex with similarity to recombinant latent TGF beta. Mol Endocrinol 1991 : 5 : 741-51.
35. Chenu C, Pfeischifter J, Mundy GR, Roodman GD. TGF-β inhibits formation of osteoclast-like cells in long-term humna bone marrow cultures. Proc Natl Acad Sci 1988 : 85 : 5683-7.
36. Pfeischifter J, Seyedin SM, Mundy GR. TGF-β 8 inhibits bone resorption in foetal rat long bone cultures. J Clin Invest 1988 : 82 : 680-5.

국문초록

쥐의 골세포에서 PGE_2 합성과 plasminogen activator 활성 조절에 의한 IL-1β의 골 흡수유도와 TGF-β에 의한 골 흡수 억제 기전에 관한 연구

경희대학교 치과대학 교정학교실

김 영 훈 · 이 영 준 · 정 규 림 · 박 영 국

골세포는 골대사에 영향을 미치는 다양한 성장인자와 싸이토카인을 생성하여 골 기질로 유리시킨다. 이 연구는 쥐의 장골 세포 배양 모델에서 recombinant human IL-1β가 PGE_2 합성과 plasminogen activator의 활성 조절을 통한 골

흡수 유도 기전의 일단을 구명하고, 이와 동시에 TGF-β에 의한 골흡수 억제 기전을 해명하는데 그 목적이 있다.

쥐의 장골 세포를 배양하여 통법의 골모세포 phenotype을 발현하는 세포를 분리하고 세포 배양능, alkaline phosphatase assay, PG assay, 골흡수능 측정들을 시행하여 다음의 결과를 얻었다.

1. IL-1β는 쥐의 골모세포의 증식, PGE_2 생성 및 palsmonogen activator의 활성을 촉진하였다.
2. IL-1β는 쥐의 골모세포에서의 alkaline phosphatase 활성을 감소시켰다.
3. rhIL-1β는 골 흡수를 촉진시켰다.
4. TGF-β는 쥐의 장골 세포에서 골의 흡수를 억제하였으며, Vitamin D_3에 의하여 유도된 골 흡수를 억제하였다.

이상의 연구 결과는 IL-1β에 의한 골 파괴의 병인과 관련하여 골 세포 대사의 병리학적 조절에 있어서의 IL-1β의 역할을 지지하며, 이와 동시에 골 흡수 억제에 있어서의 TGF-β의 역할을 확인시켜주는 것으로 생각된다.

주요 단어 : IL-1β, TGF-β, 조골세포, 파골세포 PGE_2, 골흡수