Korean Journal of Life Science
Vol. 10. No 5. 529~532, 2000

Lipopolysaccharide and Prostaglandin E_2 regulates the gene expression of IL-1β in mouse osteoblast cells

Sang-Ho Noh, Young-Guk Park* and Cheorl-Ho Kim[1]*

Department of Orthodondritics, Kyung-Hee University College of Dental Medicine, Seoul 130-701, Korea,
[1]Department of Biochemistry and Molecular Biology, College of Oriental Medicine, DongGuk University, Sukjang-Dong #707, Kyung-Ju City, Kyungpook 780-714, Korea.

Abstract

Prostaglandin E_2 (PGE_2) is an abundant eicosanoid in bone that has been implicated in a number of pathological states associated with bone loss, and is also known to stimulate matric metalloproteinase-1 synthesis and secretion in rat and human osteoblast cells, although the intracellular reaction remain unclear. Interleukin-1β (IL-1β) is a cytokine that plays a critical role in bone remodelling and appears to act as a downstream effector of most bone-resorbing agents. However, it is still interesting to examine whether PGE_2 regulates IL-1β expression by mouse osteoblasts or not. Here we demonstrate that PGE_2 is a potent inducer of IL-1β production by fetal osteoblasts.

Key Words — Prostaglandin E_2; Interleukin-1β; Osteoblast

Introduction

Prostaglandins are complex regulators of bone metabolism, being simultaneously potent stimulators of osteoclastic bone resorption and of osteoblastic bone formation [1]. Prostaglandin E_2 (PGE_2)[1] is the most abundant eicosanoid present in bone, and excess level of PGE_2 have been implicated in a number of pathological states associated with bone loss, such as hypercalcemia of malignancy, periodontal disease, and rheumatoid arthritis [2]. PGE_2 is mediator in a net anabolic effect on bone and bone matrix descruction in a variety of inflammatory processes, including fracture callus remodelling [3], rheumatoid arthritis [2], osteomyelitis [4], metastatic disease [5], periprosthetic osteolysis [6], and inflammatory diabetes [7]. To date, many of the cellular and intracellular events involved in these matrix-remodeling states remain unclear. In osteoblasts, PGE_2 augments intracellular cAMP [8] and strongly induces the production of insulin-like growth factor-I (IGF-I) [9].

There is increasing evidence indicating that the bone resorbing activity of cytokines such as IL-1β, TNF-α, and IL-6 is partially dependent on PGE_2 production [10]. Thus, it is thought that PGE_2 may act as a facilitator or enhancer of the resorptive response to cytokines and growth factors [1]. Recent study indicates that PGE_2 stimulates osteoclast formation via endogenous IL-1β [11]. The nature of the target cells responsible for the production of IL-1β in the bone environment has, however, not yet been clarified. In view of the critical role played by IL-1β in bone metabolism and of the evidence that PGE_2 induces IL-1β in the bone environment, it is interesting to address whether PGE_2 regulates IL-1β expression in mouse osteoblasts. Here we report that PGE_2 induces IL-1β production by fetal mouse osteoblasts.

*To whom all correspondence should be addressed
Tel · +82-54-770-2663. Fax . +82-54-770-2281.
E-mail · chkimbio@dongguk.ac.kr

Materials and Methods

Materials

PGE_2, PGE_1, lipopolysaccharide (LPS) from *E. coli* and BSA were purchased from Sigma Chemical Co. (St. Louis, MO). Radiochemicals were obtained from New England Nuclear Corp. (Boston, MA). Tissue culture media and reagents, Fetal bovine serum (FBS) were from Gibco (Chagrin Falls, OH). The mRNA isolation kit and DNA labelling kit were obtained from Takara Co. (Osaka, Japan) and Promega (Madison, WI). Recombinant human IL-1β was obtained from Genzyme Corp. (Cambridge, MA, USA) or our deposit as described previously [7].

Osteoblasts isolation and culture

Mouse calvarial osteoblasts were isolated from neonatal BALB/c mice by enzymatic digestion, as described for rat osteoblasts [8,9]. The population released during the last three digestions was highly enriched in cells expressing two markers of the osteoblast lineage, alkaline phosphatase and osteocalcin [12]. Cells released by collagenase digestions were washed and grown to confluent in 75 ㎠ culture flasks (Falcon) in Dulbesso's modification of Eagel's medium (DMEM) supplemented with antibiotics (penicillin and streptomycin) and 10% fetal bovine serum (FBS; Gibco, BRL, Bethesda, MD, USA). Incubations were carried out at 37℃ in a humidified atmosphere of 5% CO_2/95% air; the medium was changed every 2-3 days. Cells were grown to confluence at 37℃ and cultured in duplicate or triplicate wells for an additional 24 h in serum-free medium supplemented with Polymixin B sulfate to prevent endotoxin effects prior to treatment.

RNA isolation and Northern blot analysis

Conditioned confluent cultures were treated for the desired time periods with experimental medium alone (controls) or medium containing the agents of interest. The prostaglandins wre dissolved in absolute ethanol, except PGI_2, which was dissolved in 100 mM Tris-HCL (pH 9.4). After appropriate treatment period, cells were rinsed once with 10 ml PBS and then harvested by adding 10 ml lysis buffer directly to flasks. Poly (A)+ RNA was isolated from the lysate according to the manufacturer's instructions or using the guanidine isothiocyanate lysis method. Five micrograms of each sample were size-separated on 2.2 M formaldehyde-1.0% agarose gels. The mRNA was transferred to Zeta probe membrane or a nylon membranes (GeneScreen plus, NEN, Boston, MA, USA), and fixed by UV cross-linking. Prehybridization and hybridization with [^{32}P]deoxy-CTP-labeled complementary DNA (cDNA) probes (10^7 cpm/ml) was performed at 42℃ in 50% formamide, 5 X SSC (1 X SSC = 150 mM NaCl and 15 mM sodium citrate, pH 7.0), and 0.2% each of polyvinylpyrrolidone, BSA, and Ficoll with 250 μg/ml sheared salmon sperm DNA, 0.1% sodium dodecyl sulfate (SDS), 50 mM sodium phosphate (pH 6.5), and transfer RNA (25 μg/ml). The filters were washed in a 2 X SSC-0.1% SDS four times for 5 min at room temperature, followed by two 15-,im washes in 0.1 X SSC-0.1% SDS at 50℃. Northern blots were analyzed by autoradiography (XAR-5 film, Eastman Kodak, Rochester, NY), and the bands were quantitated by using densitometer. All data were normalized to β-actin mRNA abundance, which showed no general change in response to the prostaglandins.

Plasmids

Mouse β-actin and IL-1β gene were described previously [7].

Determination of the IL-1β amount in culture fluids

The amount of IL-1β produced by osteoblasts in culture fluids was determined by an immunoassay specific for mouse IL-1β (BioSource International).

Results and Discussion

PGE_2 stimulates the production of IL-1β in mouse calvarial osteoblasts

Osteoblasts were isolated from fetal mouse calvarial

bones by sequential collagenase digestion. The population released during the last three digestions is highly enriched in cells expressing two markers of the osteoblast lineage, alkaline phosphatase and osteocalcin [12]. We have used this osteoblast-enriched population as osteoblast cells for future study. To investigate whether PGE_2 stimulates the production of IL-1β by mouse calvarial osteoblasts, primary cultured of confluent fetal mouse osteoblasts were treated with PGE_2 for increasing amounts of time in the presence or absence of LPS, a well known stimulator of IL-1β [13], and the supernatants were analyzed for IL-1β immunoassays. As shown in **Fig. 1**, the constitutive level of IL-1β production by osteoblasts was very low, and PGE_2 induced it strongly. The induction of IL-1β production was highly sensitive and increased with time dependent. This induction of IL-1β was dose dependent and persited for 36 h of culture. When LPS was used as positive control for induction of IL-1β, it strongly stimulated the production of IL-1β with the same kinetics as PGE_2, as expected. These data indicated that PGE_2 is a potent inducer of IL-1β production by mouse calvarial osteoblasts.

To investigate the regulation of IL-1β gene expression by PGE_2, osteoblasts were stimulated for increasing amounts of time with PGE_2 or LPS and subjected to Northern hybridization analysis. As shown in **Fig. 2**, unstimulated osteoblasts expressed an undetectable level of IL-1β mRNA. Their stimulation with 0.1 μM PGE_2 for 1 h was not detectable in IL-1β mRNA expression, while 1.0 μM PGE_2 stimulated IL-1β mRNA transcription and the value of stimulation was similar to that stimualated by 20 μg LPS.

Prostaglandins are known to be potent activators of bone remodeling and have been reported as having both anabolic and catabolic effects on bone. Recent in vivo administrations have demonstrated that systemic PGE_2 results in an increase in both bone resorption and formation with a net anabolic effect and prostaglandins are also thought to have very potent resorptive effects in

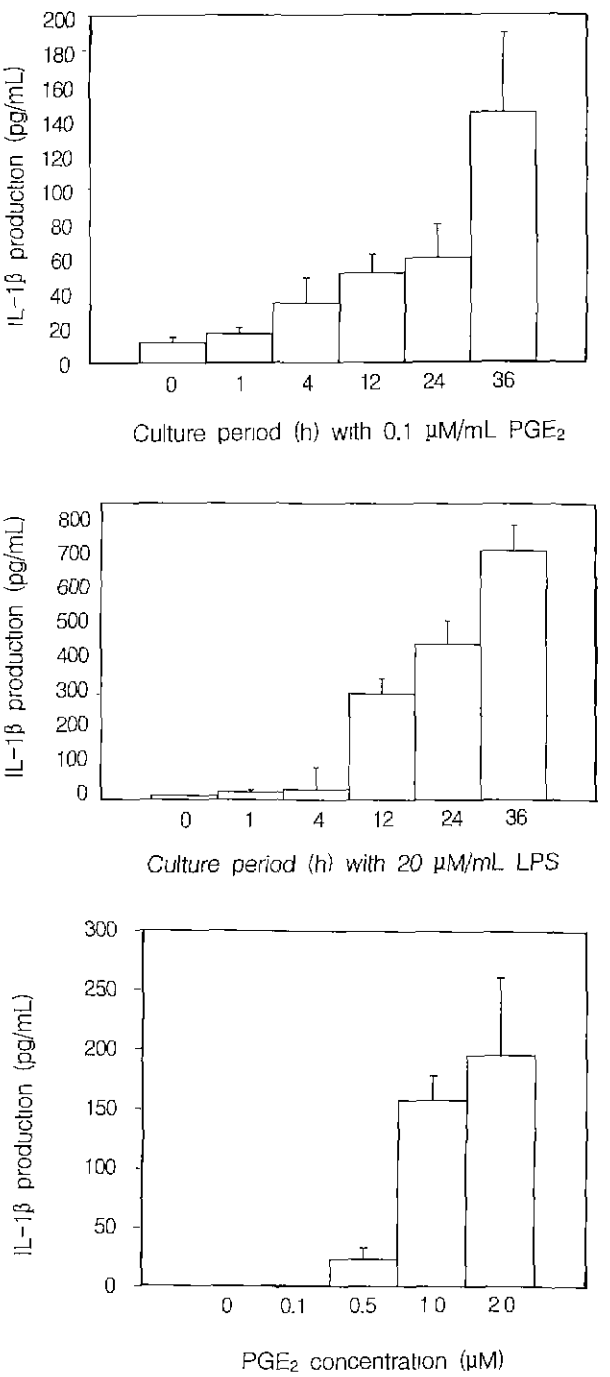

Fig. 1. Stimulating activities of PGE_2 and LPS on IL-1β production in the mouse calvarial osteoblastic cells. Confluent cells were treated with increasing amounts of time with 0.1 μM PGE_2 (A), 20 μg/ml LPS (B), or increasing amounts of PGE_2 for 4 h (C). Culture supernatants from four separate wells were harvested and tested for IL-1β amount. This figure is representative of two independent experiments which gave similar results.

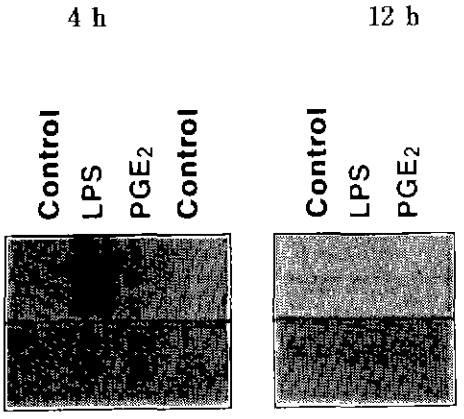

Fig. 2. Effects of PGE_2 and LPS on IL-1β mRNA transcription in the mouse calvarial osteoblastic cells. Confluent cells were cultured for increasing amounts of time with 0.1 μM PGE_2 or LPS (20 μg/ml). Total RNA was extracted and subjected to Northern hybridization. Blots were hybridized with IL-1β and β-actin probes. A representative autoradiogram after a 6-h exposure is shown. Even though the long exposures (8 and 12 hrs) were detectable, signals were undetectable in autoradiograms caused by short-term (1 and 2 hrs) treatments and low amounts (below 0.001 μM) of PGE_2.

a variety of in vivo and in vitro systems [2-4, 14]. This study addresses that PGE_2 is a strong inducer of IL-1β.

Information on the control of IL-1β gene expression by primary mouse osteoblasts is limited and the regulatory elements in PGE_2-induced IL-1β gene expression remain unclear yet. PGE_2 induced both the IL-1β production and gene expression in LPS-stimulated mouse calvarial osteoblasts.

References

1. Kawaguchi, H., C. C. Pilbeam, J. R. Harrison, and L. G. Raisz. 1995. The role of prostaglandin in the regulation of bone metabolism. *Clin. Orthop.* **313**, 36-46.
2. Robinson, D. R., A. H. Tashjian, and L. Levine. 1975. Prostaglandin-stimulated bone resorption by rheumatoid synovia. *J. Clin. Invest.* **56**, 1181-1188.
3. Dekel, S., G. Lenthal, and M. J. O. Francis. 1981. Release of prostaglandins from bone and muscle after tibial fracture. *J. Bone Joint Surg.* **63**, 185-189.
4. Dekel, S. and M. J. O. Francis. 1981. The treatment of osteomyelitis of the tibia with sodium salicylate. *J. Bone Joint Surg.* **63**, 178-184.
5. Galasko, C. S. B., and A. Bennett. 1976. Relationship of bone destruction in skeletal metastases to osteoclast activation and prostaglandins. *Nature* **263**, 508-510.
6. Goldberg, S. R., A. L. Schiller, M. Roelke, C. M. Rourke, D. A. O'Neil, and W.H. Harris, 1983. The synovial-like membrane at the bone-cement interface in loose total hip replacements and its proposed role in bone lysis. *J. Bone Joint Surg.* **65**, 575-584.
7. Park, C., J. R. Kim, J. K. Shim, B. S. Kang, Y. G. Park, K. S. Nam, Y. C. Lee, and C. H. Kim. 1999. Inhibitory effects of streptozotocin, tumor necrosis-α, and interleukin-1β on glucokinase activity in pancreatic islets and gene expression of GLUT2 and glukokinase. *Arch. Biochem. Biophys.* **362**, 217-224.
8. McCarthy, T. L., M. Centrella, and E. Canalis. 1990. Cyclic AMP induces insulin-like growth factor-1 synthesis in osteoblast-enriched cultures. *J. Biol. Chem.* **265**, 15353-15356.
9. McCarthy, T. L., M. Centrella, L. G. Raisz, and E. Canalis. 1991. Phosphoglandin E_2 stimulates insulin-like growth factor I synthesis in osteoblast-enriched cultures from fetal rat bone. *Endocrinology* **128**, 2895-2900.
10. Akatsu, T., N. Takahashi, N. Udagawa, K. Imamura, A. Yamaguchu, K. Sato, N. Nagata, and T. Suda. 1991. Role of prostaglandins in interleukin-1 induced bone resorption in mice in vitro. *J. Bone Miner. Res.* **6**, 183-190.
11. Amano, S., K. Naganuma, Y. Kawata, K. Kawakami, S. Kitano, and S. Hanazawa. 1996. Prostaglandin E_2 stimulates osteoclast formation via endogenous IL-1β expressed through protein kinase A. *J. Immunol.* **156**, 1931-1936.
12. McCarthy, T. L., M. Centrella, and E. Canalis. 1988. Further biochemical and molecular characterization of primary rat parietal bone cell cultures. *J. Bone Miner. Res.* **3**, 401-408.
13. Millet, I., T. L. McCarthy, and A. Vignery. 1998. Regulation of interleukin-6 production by prostaglandin E_2 in fetal rat osteoblasts: role of protein kinase A signaling pathway. *J. Bone Miner. Res.* **13**, 1092-1100.
14. Clohisy, J. C., T. J. Connolly, K.D. Bergman, C. O. Quinn, and N. C. Patridge. 1994. Prostanoid-induced expression of matrix mettaloproteinase-1 messenger ribonucleic acid in rat osteosarcoma cells. *Endocrinology* **135**, 1447-1453.