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Short communication

Down-regulation of interleukin-1α-induced matrix metalloproteinase-13 expression via EP_1 receptors by prostaglandin E_2 in human periodontal ligament cells

K. Noguchi[1], S. M. P. M. Ruwanpura[1], M. Yan[1,2], N. Yoshida[3], I. Ishikawa[1]

[1]Periodontology, Department of Hard Tissue Engineering, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan, [2]Department of Pediatric Dentistry, The First School of Medicine, Harbin Medical University, Harbin, China, [3]Department of Dental Hygiene, Shizuoka College, University of Shizuoka, Shizuoka, Japan

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In the present study, we investigated the effect of prostaglandin (PG) E_2 on matrix metalloproteinase (MMP)-13 production in human periodontal ligament cells stimulated with interleukin (IL)-1α. IL-1α enhanced both MMP-13 and PGE_2 production. Indomethacin, a nonselective cyclooxygenase inhibitor, and NS-398, a specific cyclooxygenase-2 (COX-2) inhibitor, significantly enhanced IL-1α-induced MMP-13 production in periodontal ligament cells, although both the agents completely inhibited IL-1α-induced PGE_2 production. Exogenous PGE_2 reduced IL-1α-induced MMP-13 mRNA and protein production in a dose-dependent manner. 17-phenyl-ω-trinor PGE_2, a selective EP_1 receptor agonist, mimicked the inhibitory effect of PGE_2 on IL-1α-induced MMP-13 mRNA and protein production. On the basis of these data, we suggest that COX-2-dependent PGE_2 down-regulates IL-1α-elicited MMP-13 production via EP_1 receptors in human periodontal ligament cells. PGE_2 may be involved in the regulation of destruction of extracellular matrix components in periodontal lesions.

Key words: EP receptors, interleukin-1α; matrix metalloproteinase-13; periodontal ligament cells; prostaglandin E_2

Kazuyuki Noguchi, Department of Hard Tissue Engineering, Graduate School, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113–8549, Japan
Tel.: +81 35803 5488;
fax: +81 35803 0196;
e-mail: kazuyuki-noguchi.peri@tmd.ac.jp
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Matrix metalloproteinases (MMPs) are a family of zinc-dependent endoproteases that degrade extracellular matrix (1). MMP-13 (collagenase-3) cleaves fibrillar collagens with a preference for type II collagen over types I and III, and have 40-fold stronger gelatinase activity than MMP-1 (collagenase-1) and MMP-8 (collagenase-2). In addition to fibrillar type I, II and III collagens, it degrades type IV, IX, X and XIV collagens, gelatin, tenacin-C, fibronectin and proteoglycan core proteins (4, 5, 9, 10). It has been demonstrated that metalloproteinases including MMP-13 are present in gingival tissue explants and that there is an inverse correlation between the loss of collagen fibers and the increase of MMP-13 levels (3). Gingival crevicular fluid proMMP-13 levels have been shown to be significantly correlated with the gingival and bleeding indices (7). Furthermore, it has been suggested that several types of MMPs, including MMP-13 (collagenase-3), play a crucial role in destruction of periodontal extracellular molecules (28). Thus, it is very likely that MMP-13 is involved in the pathogenesis of periodontal disease.

Prostaglandins (PGs) play important roles in the regulating of diverse cellular functions under physiologic and pathologic conditions (2). In response to stimuli, including interleukin (IL)-1, arachidonic acid released from membrane phospholipid is metabolized to several types of PGs including PGE_2 by cyclooxygenase

(COX). Two isoforms of COX, COX-1 and COX-2, have been identified (2, 25). Whereas COX-1 is expressed in many tissues, COX-2 is induced after stimulation by cytokines, growth factors, and lipopolysaccharide and is recognized as an immediate early gene. It is believed that PGs, including PGE_2, are involved in the pathogenesis of periodontal disease (20). Non-steroidal anti-inflammatory drugs (NSAIDs) which inhibit PG synthesis have been reported to prevent periodontal destruction. In particular, PGE_2 has been shown to be a potent stimulator of bone resorption and to be associated with attachment loss (8, 21, 24). Previously, we have demonstrated that COX-2 is responsible for PGE_2 production in human periodontal ligament cells challenged with IL-1α (19). However, the role of COX-2-dependent PGE_2 in periodontal ligament cells remains unclear.

Recent studies have demonstrated that the cellular effects of PGE_2 are mediated by the activation of multiple functionally distinct subtypes of PGE_2 receptors. These are divided into EP_1, EP_2, EP_3 and EP_4, based on their ligand-binding selectivities and signaling pathway mechanisms (14, 15). EP_1 receptors are coupled to intracellular Ca^{2+} signaling. EP_2 and EP_4 receptors activate adenylate cyclases via a cholera toxin-sensitive, stimulatory G protein and elevate intracellular cAMP levels. EP_2 receptors are sensitive to butaprost, an agent that selectively binds PGE_2 receptors, whereas EP_4 receptors are not. Multiple isoforms of EP_3 receptors with different C-terminal tails are generated by alternative mRNA splicing. EP_3 receptor variants mediate several signaling pathways including inhibition and stimulation of adenylate cyclase, activation of phospholipase C and mobilization of intracellular calcium.

Recently, it has been demonstrated that MMP-13 gene is expressed during tooth movement in rats and that PGE_2 down-regulates tumor necrosis factor (TNF) α-induced MMP-13 production in human periodontal ligament cells (16, 26). In the present study, we investigated the effect of PGE_2 on MMP-13 production in human periodontal ligament cells stimulated with IL-1α. Furthermore, we analyzed which EP receptors were involved in PGE_2 regulation of IL-1α-induced MMP-13 production.

Human periodontal ligament cells were obtained from explants of periodontal ligaments from premolars or third molars extracted from periodontally healthy subjects. Informed consent was obtained from all subjects. The study protocol was approved by the Ethical Committee of the Tokyo Medical and Dental University. The cells were grown in α-Minimum Essential Medium (α-MEM) supplemented with 10% fetal bovine serum (Bioserum, Victoria, Australia), 100 U/ml penicillin (Sigma Chemical Co., St. Louis, MO), and 100 U/ml streptomycin (Sigma Chemical Co.), and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Cells used for the experiments were between the 5th and 10th passages. Periodontal ligament cells were seeded in 96-well plates for assay of MMP-13 and PGE_2 levels in culture media and in 6-well plates for reverse transcription-polymerase chain reaction (RT-PCR) assay of MMP-13 mRNA, respectively. After confluence, they were serum-starved in α-MEM containing 0.5% fetal bovine serum to reduce the effect of serum on MMP-13 production. After 24 h, the cells were treated with IL-1α (Sigma Chemical Co.), PGE_2 (Cascade Biochem LTD, Berkshire, UK), 17-phenyl-ω-trinor PGE_2 (Cayman Chemicals, Ann Arbor, MI), butaprost, ONO-AP-324, ONO-AE1-329 (gifts from ONO Pharmaceuticals Co. Ltd, Tokyo, Japan), indomethacin and NS-398 (Wako, Tokyo, Japan) in combinations and concentrations indicated. For RT-PCR of MMP-13 mRNA, 24 h after treatment, total RNA was extracted by guanidinium thiocyanate/phenol/chloroform method, using ISOGEN (Nippon Gene, Toyama, Japan). cDNA primers were synthesized using 2 μg of total RNA reverse transcriptase and oligo (dT) primers (Takara Co., Shiga, Japan). The specific primer pairs for human MMP-13 and β-actin were selected according to previous reports (4, 22, 23). The primers were MMP-13: sense primer 5′-CATTTGATGGGCCCTCTGGCCTGC-3′, antisense primer 5′- GTTTAGGGTTGGGGTCTTCATCTC-3′, β-actin: sense primer 5′-GTGGGCATGGGTCATCAGAAGGAT-3′, antisense primer: 5′-CTCCTTAATGTCACGCACGATTTC-3′. PCR reaction was performed in 25 μM of each primer, 2.5 mM of each dNTP and 2.5 units of Taq DNA polymerase (Takara Co.) in an automated DNA thermal cycler. The PCR amplification comprised 28 cycles for MMP-13 of denaturation at 96°C for 30 s, annealing at 55°C for 1 min and extension at 72°C for 2 min. For β-actin, 28 cycles comprising denaturation at 96°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 3 min the RT-PCR products were resolved by electrophoresis in 1.5% agarose gel containing ethidium bromide. The intensities of obtained bands were determined using SYGENE Bio Imaging System (SYGENE, Cambridge, UK). For assay of MMP-13 and PGE_2 levels, culture media were collected 72 h after cells were treated as described above, and MMP-13 and PGE_2 levels in culture media were analyzed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Amersham Pharmacia Biotech, Buckinghamshire, UK). ELISA data are expressed as the mean ± standard deviation (SD). Data were subjected to 1-way analysis of variance (ANOVA), using the StatView program. Fisher's protected least significance test was used in the *post hoc* comparison of specific groups.

It has been demonstrated *in vivo* that MMP-13 levels are enhanced in periodontal lesions and that several types of cells including gingival fibroblasts and sulcular epithelium express MMP-13 (28). As shown in Fig. 1A and 1B, IL-1α induced MMP-13 mRNA and protein expression in periodontal ligament cells. It has been demonstrated that TNFα induces MMP-13 mRNA and protein expression in periodontal ligament cells (16). Thus, it is likely that periodontal ligament cells are one of MMP-13-producing cell types.

Previously, we have demonstrated that IL-1α produces PGE_2 via COX-2 in periodontal ligament cells (19). In order to clarify the involvement of COX-2-derived PGE_2 in MMP-13 expression, periodontal ligament cells were treated with indomethacin, a nonselective COX inhibitor and NS-398, a selective COX-2 inhibitor. Both agents significantly augmented IL-1α-induced MMP-13 mRNA and protein expression to the similar extent, although IL-1α-induced PGE_2 production was completely suppressed (Fig. 1A–C). We examined the effect of exogenous PGE_2 on IL-α-induced MMP-13 production in periodontal ligament cells. As indicated in Fig. 2A and B, PGE_2 dose-dependently reduced IL-1α-induced MMP-13 mRNA and protein expression. From these data, we suggest that IL-1α-induced MMP-13 production is regulated by endogenous PGE_2 generated via COX-2 in periodontal ligament cells. Finally, we investigated which EP receptors were involved in PGE_2 regulation of IL-1α-induced MMP-13 expression using various specific EP agonists. As demonstrated in Fig. 3A and B, 17-phenyl-ω-trinor PGE_2, a selective EP_1 agonist, mimicked the inhibitory effect of PGE_2, although butaprost, a selective EP_2 agonist, and ONO-AE1-329, a selective EP_4 agonist, could not cause significant effects. ONO-AP-324, a selective EP_3 agonist, had no effect on IL-1α-induced

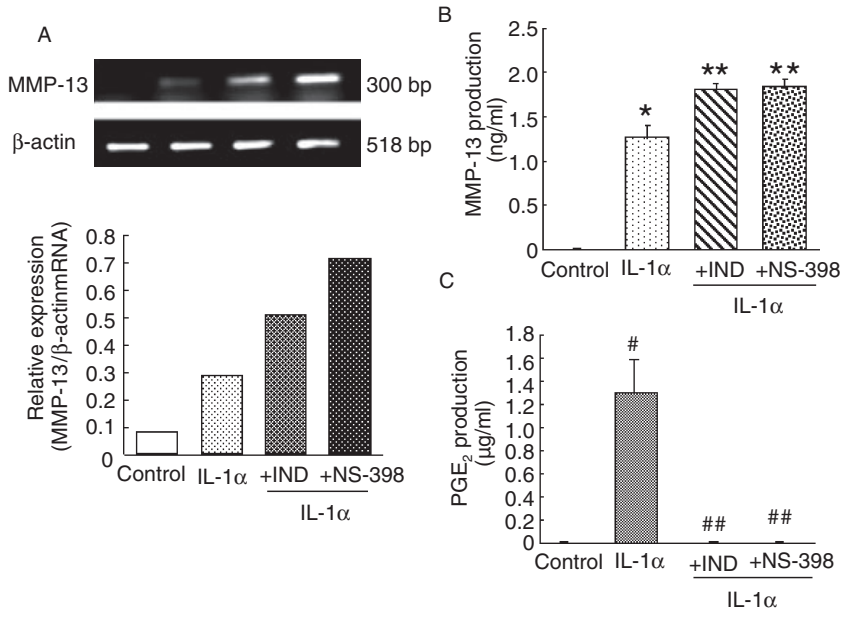

Fig. 1. Effect of indomethacin and NS-398 on MMP-13 mRNA expression (A), MMP-13 (B) and PGE_2 levels (C) in IL-1α-stimulated periodontal ligament cells. A) Periodontal ligament cells were stimulated with vehicle or 2 ng/ml IL-1α in the presence or absence of 1 μM indomethacin (IND) and 1 μM NS-398 for 24 h. After incubation, total RNA was extracted and MMP-13 mRNA expression was examined by RT-PCR. The ratio of MMP-13 mRNA expression to β-actin mRNA expression was calculated using SYGENE Bio Imaging System. B and C) Periodontal ligament cells were stimulated with vehicle or 2 ng/ml IL-1α in the presence or absence of 1 μM indomethacin (IND) and 1 μM NS-398 for 72 h. After incubation, MMP-13 (B) and PGE_2 (C) levels in the culture media were evaluated by ELISA. Values are mean ± SD ($n = 4$). Data are representative of three separate experiments. B: *Significantly different from control ($P < 0.0001$). **Significantly different from IL-1α alone ($P < 0.0001$). C: #Significantly different from control ($P < 0.0001$). ##Significantly different from IL-1α alone ($P < 0.0001$).

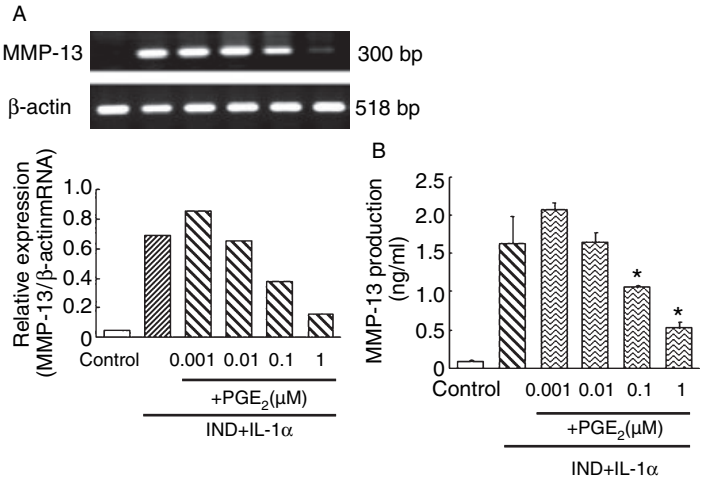

Fig. 2. Effect of PGE_2 on MMP-13 mRNA and protein expression in IL-1α-stimulated periodontal ligament cells. A) Periodontal ligament cells were stimulated with vehicle or 2 ng/ml IL-1α in the presence of 1 μM indomethacin (IND) and various doses of PGE_2 for 24 h. After incubation, total RNA was extracted and MMP-13 mRNA expression was examined by RT-PCR. The ratio of MMP-13 mRNA expression to β-actin mRNA expression was calculated using SYGENE Bio Imaging System. B) Periodontal ligament cells were stimulated with vehicle or 2 ng/ml IL-1α in the presence of 1 μM indomethacin (IND) and various doses of PGE_2 for 72 h. After incubation, MMP-13 levels in the culture media were evaluated by ELISA. Values are mean ± SD ($n = 4$). Data are representative of three separate experiments. *Significantly different from control ($P < 0.0001$). **Significantly different from IL-1α alone ($P < 0.0001$).

MMP-13 expression (data not shown). These results suggest that PGE_2 regulation of IL-1α-induced MMP-13 expression is mediated via EP_1 receptors. It has been shown that TNFα-induced MMP-13 production is inhibited by cAMP in periodontal ligament cells (16). However, butaprost and ONO-AE1-329 could not affect IL-1α-induced MMP-13 expression, suggesting that IL-1α-induced MMP-13 production is not regulated by cAMP-dependent pathways through EP_2 and/or EP_4 receptors. It is likely that TNFα- and IL-1α-induced MMP-13 production is differently regulated in periodontal ligament cells. Further studies are necessary to elucidate the mechanism by which PGE_2 inhibits IL-1α-induced MMP-13 expression via EP_1 receptors in periodontal ligament cells.

In the present study, the roles of MMP-13 were not investigated in periodontal lesions. MMP-13 has been detected in gingival crevicular fluid and gingival tissues from patients with adult and localized juvenile periodontitis (6, 7, 28). It has been shown that MMP-13 is involved in collagen breakdown in periodontitis (3, 6, 29). It has been demonstrated that there is the possible association of vertical bone loss and elevated MMP-13 levels in peri-implant sulcus fluid and that MMPs including MMP-13 are associated with bone resorption in mouse calvaria (11, 12). Therefore, it is very likely that MMP-13 is involved in the pathogenesis of periodontal disease. In the present study we showed that PGE_2 inhibited IL-1α-induced MMP-13 production in periodontal ligament cells. PGE_2 has been shown to be a potent stimulator of bone resorption and to be associated with attachment loss (8, 21, 24), and is therefore thought to mediate proinflammatory and tissue-destructive responses in periodontal lesions. However, several studies have demonstrated that PGE_2 suppresses the production of proinflammatory molecules, including intercellular adhesion molecule-1, IL-6, chemokines and TNFα (13, 17, 18, 27). Together with our data that PGE_2 inhibited IL-1α-induced MMP-13 production in periodontal ligament cells, we suggest that PGE_2 may exert anti-inflammatory effects in periodontal lesions. Further investigation is required to define the roles of PGE_2 in periodontal disease.

In conclusion, we suggest that COX-2-dependent PGE_2 can down-regulate IL-1α-elicited MMP-13 production via EP_1 in human periodontal ligament cells. PGE_2 may be involved in the regulation of destruction of extracellular matrix components in periodontal lesions.

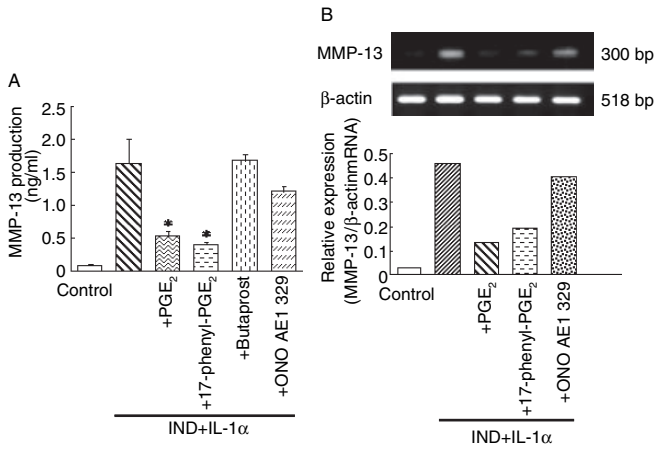

Fig. 3. Effect of 17-phenyl-ω-trinor PGE_2, butaprost and ONO-AE1-329 on MMP-13 mRNA and protein expression in IL-1α-stimulated periodontal ligament cells. A) Periodontal ligament cells were stimulated with vehicle or 2 ng/ml IL-1α in the presence of 1 μM indomethacin (IND) and 1 μM 17-phenyl-ω-trinor PGE_2, butaprost and ONO-AE1-329 for 72 h. After incubation, MMP-13 levels in the culture media were measured by ELISA. Values are mean ± SD ($n = 4$). Data are representative of three separate experiments. *Significantly different from IND + IL-1α ($P < 0.05$), **Significantly different from IND + IL-1α ($P < 0.0005$). B) Periodontal ligament cells were stimulated with vehicle or 2 ng/ml IL-1α in the presence of 1 μM indomethacin (IND) and 1 μM 17-phenyl-ω-trinor PGE_2, butaprost and ONO-AE1-329 for 24 h. After incubation, total RNA was extracted and MMP-13 mRNA expression was examined by RT-PCR. The ratio of MMP-13 mRNA expression to β-actin mRNA exprssion was calculated using SYGENE Bio Imaging System.

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