J Periodont Res 2004; 39; 168–174
Printed in the UK. All rights reserved

Copyright © Blackwell Munksgaard Ltd

JOURNAL OF PERIODONTAL RESEARCH
doi: 10.1111/j.1600-0765.2004.00720.x

Examination of the signal transduction pathways leading to activation of gelatinolytic activity by interleukin-1α and *Porphyromonas gingivalis* in human osteosarcoma cells

Yu-Chao Chang[1], Shu-Chen Chu[2], Shun-Fa Yang[3], Yih-Shou Hsieh[3], Li-Chiu Yang[4], Fu-Mei Huang[4]

[1]Department of Periodontics, Institute of Stomatology, Chung Shan Medical University, [2]Department of Food Science, Chungtai Institute of Health Sciences and Technology, [3]Institute of Biochemistry, Chung Shan Medical University and [4]Oral Medicine Center, Chung Shan Medical University Hospital, Taichung, Taiwan

Chang Y-C, Chu S-C, Yang S-F, Hsieh Y-S, Yang L-C, Huang F-M. Examination of the signal transduction pathways leading to activation of gelatinolytic activity by interleukin-1α and Porphyromonas gingivalis *in human osteosarcoma cells. J Periodont Res 2004; 39; 168–174. © Blackwell Munksgaard, 2004*

Background: Recently, evidence show that matrix metalloproteinases (MMP) play an important role in the pathogenesis of periodontal diseases. However, the mechanisms and signal transduction pathways involved in the production of MMPs in human osteosarcoma cells are not fully understood.

Objectives: The purpose of this study was to investigate the gelatinolytic activity in human osteosarcoma cells stimulated with interleukin-1α (IL-1α) or *Porphyromonas gingivalis* in the absence or presence of SB203580 (p38 inhibitor), U0126 [mitogen-activated protein kinase kinase (MEK) inhibitor], and LY294002 [phosphatidylinositaol 3-kinase (PI3K) inhibitor].

Methods: IL-1α and the supernatants of *P. gingivalis* were used to evaluate gelatinolytic activity in human osteosarcoma cells using gelatin zymography. Furthermore, to search possible signal transduction pathways, SB203580, U0126, and LY294002 were added to test how they modulated the gelatinolytic activity.

Results: Gelatin zymography demonstrated that the latent proforms of gelatinases MMP-2 and MMP-9 were released by human osteosarcoma cells. Secretion of MMP-9 was time-dependent by stimulating with IL-1α or *P. gingivalis*. In addition, SB203580, U0126, and LY294002 significantly reduced the IL-1α or *P. gingivalis*-stimulated MMP-9 production, respectively ($p < 0.05$). However, none of the kinase inhibitors affected the MMP-2 level compared with the control during the 4-day culture period ($p > 0.05$).

Conclusions: Our findings demonstrated that IL-1α and *P. gingivalis* enhance MMP-9 production in human osteosarcoma cells, and the signal transduction

Dr Fu-Mei Huang, Oral Medicine Center, Chung Shan Medical University Hospital, 110, Sec. 1, Chien-Kuo N. Road, Taichung, Taiwan.
Tel: +886 4 24718668 ext. 5128
Fax: +886 4 24759065
e-mail: hfm0118@csmu.edu.tw

Key words: gelatin zymography; human osteosarcoma cells; matrix metalloproteinases; signal transduction pathway

Accepted for publication October 21, 2003

pathways p38, MEK, and PI3K are involved in the inhibition of MMP-9. SB203580, U0126, and LY294002 suppress MMP-9 production and/or activity and may therefore be valuable therapeutics in MMP-mediated periodontal destruction, and might be proved clinically useful agents, in combination with standard treatment modalities, in the treatment of periodontitis.

Matrix metalloproteinases (MMP), a family of host-derived enzymes that includes the collagenases and the gelatinases, are intimately involved in the degradation and remodeling of extracellular matrix protein during different developmental processes such as organ morphogenesis, bone formation, angiogenesis, and remodeling during reproductive processes, as well as in pathologic processes such as inflammation, chronic degenerative diseases and tumor invasion (1–3). The MMP family is divided into six main groups – collagenase, stromelysin, gelatinase, matrilysin, elastase and membrane-bound MMP – depending on their substrate specificity for extracellular matrix proteinase (4).

Periodontitis is an inflammatory disease accompanied by reduction of gingival tissue, destruction of periodontal ligament, alveolar bone resorption and possibly loss of teeth (5, 6). MMPs are produced by both infiltration and resident cells of the periodontium. It has been shown that an imbalance between activated MMPs and their host-derived endogenous inhibitors leads to pathological breakdown of the extracellular matrix and basement membranes during periodontitis (7). These enzymes are particularly responsible for degradation of the collagen fibers attached to the root surface, allowing for the apical migration and lateral extension of the pocket epithelium (8).

Interleukin-1 (IL-1) has a central role in the regulation of immunological and inflammatory reactions. Biological activity of IL-1 molecules seems to be directly relevant to periodontal destruction, such as periodontal attachment loss, destruction of collagen and alveolar bone resorption (9). *Porphyromonas gingivalis* is one of the predominant periodontal pathogens, and possesses multiple virulence factors and can trigger a variety of host cells to release inflammatory mediators such as inflammatory cytokines, prostaglandin and MMPs (10, 11). The differentiation and activity of the bone-resorbing osteoclasts and the release of MMPs by osteoclasts as well as osteoblasts participate in the destruction of bone (12).

MMP-2 and MMP-9, sometimes referred to as type IV collagenase and gelatinase, respectively, are of particular interest because they have been implicated in the pathogenesis of periodontitis (13–15). Our previous studies have shown that MMP-2 was up-regulated by *P. gingivalis* in human periodontal ligament fibroblasts (16) and pulp fibroblasts (17), and by IL-1α (16) in human periodontal ligament fibroblasts. However, the production and signal transduction pathways of MMPs in the predominant cell type in alveolar bone, the osteoblasts, are poorly understood. The aim of this study was therefore to investigate the production and signal transduction involved in the production of gelatinases in human osteosarcoma cells (U2OS) by gelatin zymography.

Material and methods

Chemicals and materials

IL-1α was purchased from Sigma Chemical Co. (St. Louis, MO, USA). SB203580 (p38 inhibitor), U0126 [a specific inhibitor of mitogen-activated protein kinase kinase 1/2 (MEK 1/2) which inhibits extracellular signal-regulated kinase 1/2 (ERK 1/2)], and LY294002 [phosphatidylinositol 3-kinase (PI3K) inhibitor] were obtained from Promega (Madison, WI, USA). All culture materials were obtained from Gibco (Grand Island, NY, USA). IL-1α was directly dissolved in the culture medium. Other kinase inhibitors were first dissolved in dimethyl sulfoxide and then diluted with the culture medium. The final concentration of solvent in the medium did not exceed 0.25% (v/v). At these concentrations the solvents used were not cytotoxic to human osteoblastic cells. The final concentrations of IL-1α, SB203580, U0126, and LY294002 used in this study were 10 ng/ml, 26 μM, 23 μM, and 163 μM, respectively.

Bacterial strain and preparation of supernatant

The strain tested, *P. gingivalis* (ATCC 33277), came from culture collections and was maintained in Brain Heart Infusion broth, prereduced anaerobically sterilized and supplemented with hemin (5 mg/l) and menadione (0.5 mg/l) for obligate anaerobes. The density of the inoculum, prepared in Brain Heart Infusion broth, was adjusted to turbidity of 2 McFarland standard (6×10^8 CFU/ml). After centrifugation, supernatants were filter-sterilized using a 0.2 μm filter and stored at −80°C until used. The supernatants of *P. gingivalis* were directly diluted in culture medium and the final concentration of dilution was 1 : 1000.

Cell culture

U2OS cells (ATTC HTB 96), derived from human osteogenic sarcoma, were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 μg/ml of streptomycin, and 100 mg/ml of penicillin at 37°C in a humidified incubator under ambient pressure air atmosphere containing 5% CO_2 (18). Confluent cells were detached with 0.25% trypsin and 0.05% EDTA for 5 min, and aliquots of separated cells were subcultured. The cells were subcultured at 1 : 4 splits every third day.

Drugs and bacterial supernatant treatment

Confluent cells were trypsinized, counted, and plated at a concentration of 5×10^4 cells in a 60-mm culture dish and allowed to achieve confluence. Cells were cultured for 48 h, at which time the medium was changed to a medium containing 10% fetal calf serum with or without the inflammatory mediators IL-1α or *P. gingivalis* in the absence or presence of SB203580, U0126, and LY294002. After different periods of time (days 1, 2, and 4), the conditioned medium samples were withdrawn and stored at −20°C before analysis. Finally, cytosol fractions were also collected for this experiment. The cells were extracted at 4°C for 10 min in phosphate-buffered saline containing 2% Triton X-100.

Gelatin zymography

Gelatin zymography allows the observance of enzyme activity utilizing routine sodium dodecyl sulfate–polyacrylamide gel electrophoresis. A substrate is copolymerized within the gel, the samples electrophoresed, the gel incubated at 37°C to allow for enzyme function, and then stained. The location of migrated enzyme that can degrade the incorporated substrate is visualized as a clearance zones within the stained gel. Enzyme activity is seen as the clear bands within the dark staining gel. Briefly, gelatinase activity was assayed by zymography (19) in 1.5 mm of 7.5% sodium dodecyl sulfate–polyacrylamide gel impregnated with 1 mg/ml of gelatin that had been fluorescently labeled with 2-methoxy-2,4-diphenyl-3[2H] furanone by the method of O'Grady *et al.* (20). This method allows for visual monitoring of the lysis of gelatin under long-wave UV light during incubation. In this study a 2 μl sample was electrophoresed on a 10% sodium dodecyl sulfate–polyacrylamide gel copolymerized with 2% gelatin as substrate. After electrophoresis the gels were washed in 2.5% Triton X-100 twice for 30 min to remove all sodium dodecyl sulfate. The gels were then incubated in 50 mmol/l Tris (pH 7.5), 5 mmol/l $CaCl_2$, and 1 mmol/l $ZnCl_2$ at 37°C overnight. The gelatin cleavage rate was analyzed from the photographed gels with a densitometer (AlphaImager 2000; Alpha Innotech, San Leandro, CA, USA).

Statistical analysis

Triplicate or more separate experiments were performed throughout this study. The significance of the results obtained from control and treated groups was statistically analyzed by the paired Student *t*-test. A *p*-value of < 0.05 was considered to be statistically significant.

Results

Specific characterization of MMPs in the conditioned medium by gelatin zymography demonstrated that the latent proforms of gelatinases MMP-2 (pro-MMP-2, 72 kDa) and MMP-9 (pro-MMP-9, 92 kDa) were released by U2OS cells (Figs 1 and 2). In addition, pro-MMP-2 was released in much higher amounts than pro-MMP-9. Secretion of pro-MMP-9 was time-dependent during the 4-day culture period by treatment with IL-1α (Fig. 1) or *P. gingivalis* (Fig. 2).

The involvement of signal transduction pathways participating in the regulation of MMP-2 and MMP-9 production in U2OS cells were studied by using different kinase inhibitors of p38, MEK, and PI3K.

The conditioned medium samples from U2OS cells stimulated with IL-1α in the presence of different pharmacological agents during the 4-day culture period showed the level of MMP-9 to be significantly inhibited by SB203580,

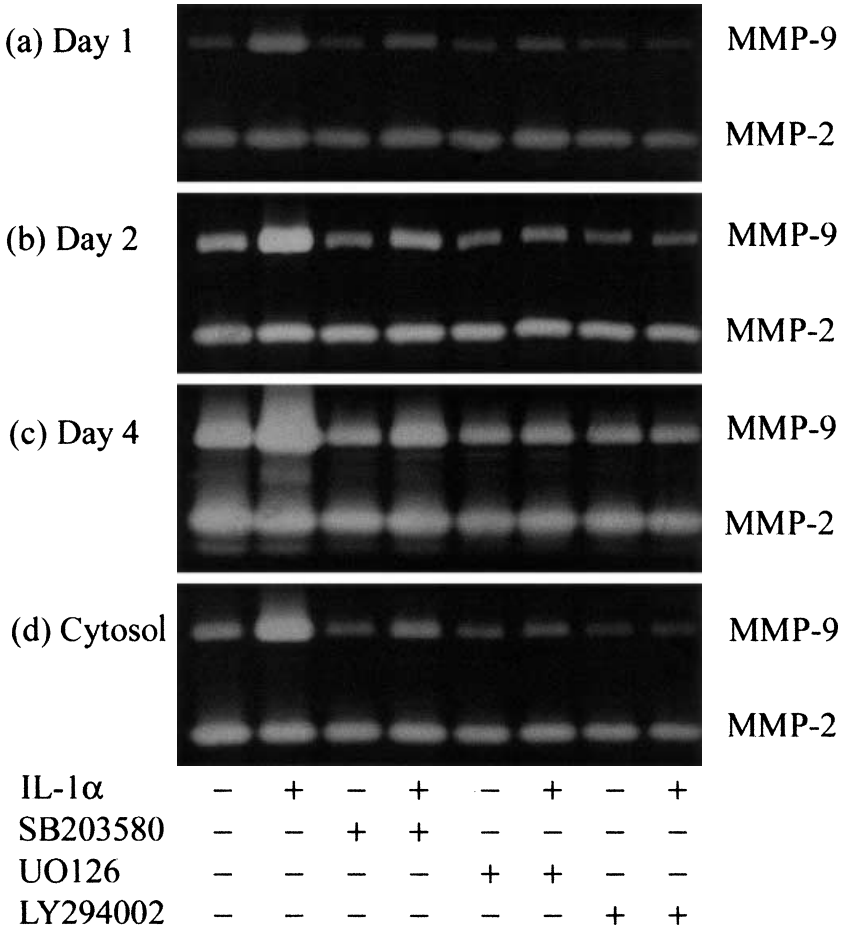

Fig. 1. Gelatin zymogram of conditioned medium (a, b, and c) and cytosol (d) from U2OS cells treated with interleukin-1α (IL-1α) in the presence of different kinase inhibitors during 4-day culture period.

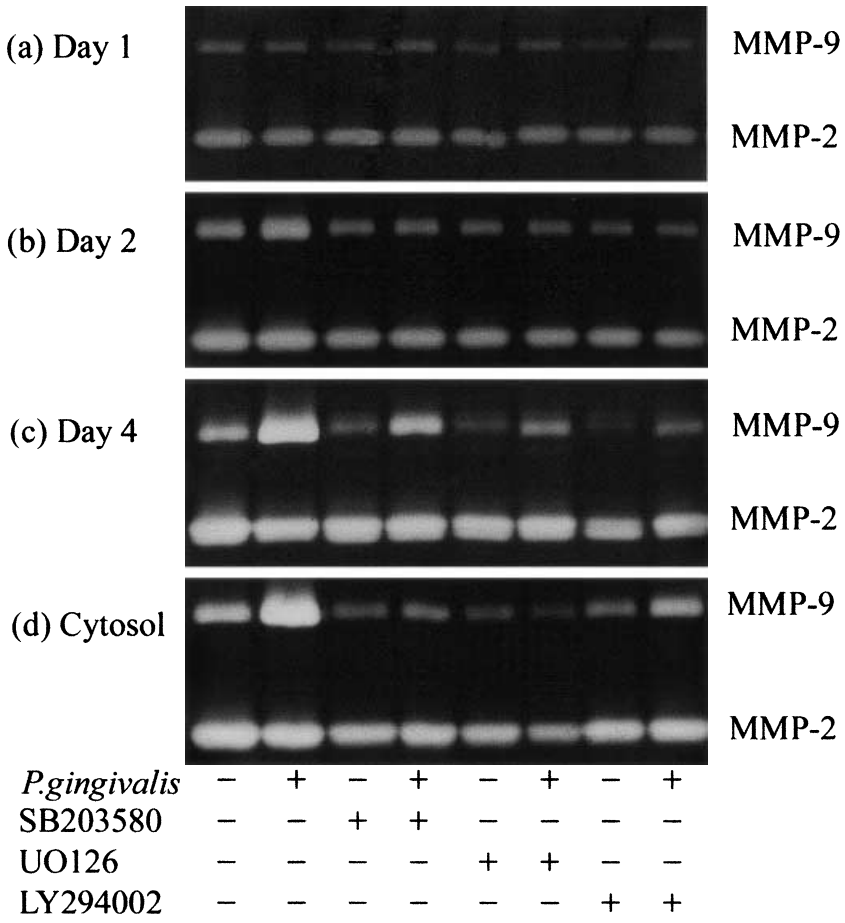

Fig. 2. Gelatin zymogram of conditioned medium (a, b, and c) and cytosol (d) from U2OS cells treated with *Porphyromonas gingivalis* in the presence of different kinase inhibitors during 4-day culture period.

U0126, and LY294002 (Fig. 1). The inhibitory pattern was shown in a time-dependent manner ($p < 0.05$). The quantitative measurements by the AlphaImager 2000 confirm these results (Fig. 3, Table 1).

The conditioned medium samples from U2OS cells stimulated with *P. gingivalis* in the presence of different pharmacological agents are shown in Fig. 2. However, none of the kinase inhibitors affected MMP-9 level compared with control during the 1-day culture period ($p > 0.05$). The conditioned medium samples from day 2 to day 4 showed that SB203580, U0126, and LY294002 significantly depressed MMP-9 synthesis (Fig. 2). In addition, the inhibition of MMP-9 was also shown in a time-dependent manner ($p < 0.05$). The quantitative measurements by the AlphaImager 2000 are shown in Fig. 4 and Table 2.

However, no drugs affected the MMP-2 level compared with control during the 4-day culture period ($p > 0.05$) (Figs 1 and 2). A gradual increase in the concentration of MMP-2 during 4-day culture was seen. This phenomena is an increase in the total cell number as the culture period progress.

We also determined the homogenate of MMP-2 and MMP-9 in U2OS cells. Similar to the conditioned medium level of MMP-9, the cytosol levels of MMP-9 were increased significantly by IL-1α (Fig. 1) and *P. gingivalis* (Fig. 2), respectively. In addition, all kinase inhibitors significantly reduced the IL-1α or *P. gingivalis*-stimulated MMP-9 production (Figs 1 and 2). The quantitative measurement of MMP-9 activity by the AlphaImager 2000 from day 1 to day 4 is shown in Figs 3 and 4. Moreover, the intracellular levels were lower (approximately 2–9 times) compared with the extracelluar levels.

Discussion

MMPs play a central role in the turnover of extracellular components. They are expressed at low levels in the absence of inflammation, wounding, or other pathological processes (1). Primary osteoblasts and osteoblastic cells are known to synthesize and secrete MMP-2 and MMP-9 (21, 22). In this study, human osteosarcoma cell line U2OS cells have been demonstrated to produce primarily MMP-2 and MMP-9. Furthermore, we demonstrated here that U2OS cells constitutively produce gelatinases, and that the production of MMP-9 is enhanced by IL-1α and *P. gingivalis*. These results were different from that of the previous studies that MMPs were up-regulated by IL-1α in human pulp fibroblasts (16), periodontal ligament fibroblasts (16, 17), and osteoblastic cells (23). In addition, MMPs was found to be elevated by *P. gingivalis* in human pulp fibroblasts (16) as well as periodontal ligament fibroblasts (17) *in vitro*.

The levels of MMP-2 and MMP-9 have been reported to be elevated in gingival crevicular fluids as well as gingival tissues from patients with periodontitis (13–15). However, the mechanisms and signal transduction pathways involved in the production of MMPs in U2OS cells are not fully understood. The novel findings of this study are that the signal transduction pathways p38, MEK, and PI3K are involved in the production of gelatinases in human osteoblastic cells. In addition, we also demonstrated that gelatinases were detected not only extracellularly but also intracellularly, although the intracellular levels were lower.

The c-Jun N-terminal kinases (JNKs) and p38 kinases constitute together with ERKs the family of mitogen-activated protein kinases (MAPKs). Phosphorylation of ERK is catalyzed by a dual specificity kinase termed MAPK kinase (MEK). MAPKs are a unique family of serine/threonine kinases that are acti-

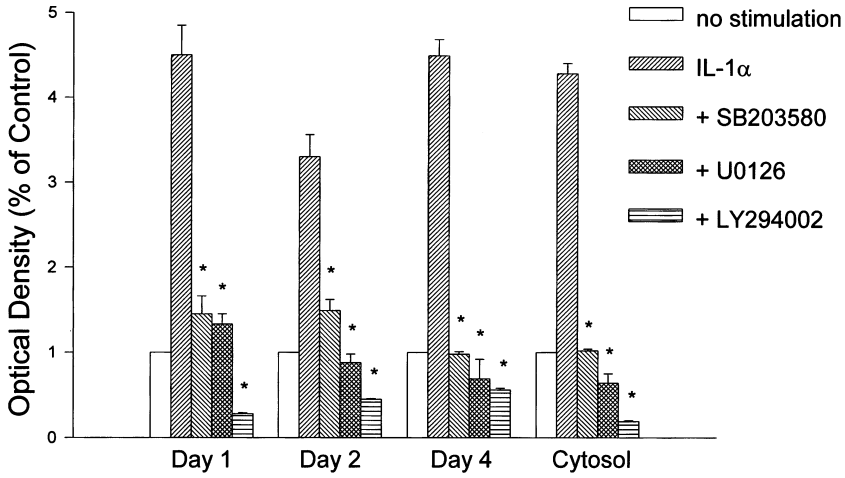

Fig. 3. Levels of matrix metalloproteinase-9 from conditioned medium and cytosol treated with interleukin-1α (IL-1α) in the presence of different kinase inhibitors were calculated from their gelatinolytic activity, as measured by AlphaImager 2000. Values are means and standard deviations of optical density from triplicate independent experiments. *Statistically significant between IL-1α alone and IL-1α added with different kinase inhibitors, $p < 0.05$. SB203580, p38 inhibitor; U0126, mitogen-activated protein kinase kinase (MEK) inhibitor; LY294002, phosphatidylinositaol 3-kinase (P13K) inhibitor.

Table 1. The effects of SB203580 (p38 inhibitor), U0126 [mitogen-activated protein kinase kinase (MEK) inhibitor], and LY294002 [phosphatidylinositaol 3-kinase (P13K) inhibitor] in the presence of interleukin-1α (IL-1α) on matrix metalloproteinase-9 activity, as measured by AlphaImager 2000. Values are means and standard deviations of optical density (percentage of control)

	IL-1α	+ SB203580	+ U0126	+ LY294002
Day 1	4.50 ± 0.35[a]	1.45 ± 0.21[b]	1.33 ± 0.12[b]	0.28 ± 0.01[b]
Day 2	3.30 ± 0.26[a]	1.49 ± 0.13[b]	0.88 ± 0.10[b]	0.45 ± 0.01[b]
Day 4	4.49 ± 0.19[a]	0.98 ± 0.03[b]	0.69 ± 0.23[b]	0.56 ± 0.02[b]
Cytosol	4.28 ± 0.12[a]	1.02 ± 0.02[b]	0.64 ± 0.11[b]	0.19 ± 0.01[b]

[a]Statistically significant in comparison with control, $p < 0.05$.
[b]Statistically significant between IL-1α alone and IL-1α + inhibitors, $p < 0.05$.

vated via reversible phosphorylation and mediate signal transduction for multiple extracellular stimuli. The MAPK families regulate a number of transcription factors, with subsequent activation of MMP and cytokine gene expression. Recently, p38 was found to play an important role in phorbol ester-induced gelatinase production by a squamous cell carcinoma cell line (24). ERK has been implicated in the regulation of collagenase gene expression in cultured fibroblasts (25). Our data also demonstrated that MAPK inhibitors down-regulated the gelatinases activity in human osteoblastic cells. Moreover, MAPK inhibitors SB203580 and U0126 were found to down-regulate the IL-1α up-regulated MMP-9 production in this study. These results are in line with Barchowsky *et al.* (26), who demonstrated that the IL-1 induced MMP-1 gene expression in synovial fibroblasts requires activation of MAPK, and Domeij *et al.* (27) who found SB203580 inhibited IL-1 up-regulation MMP-1 and MMP-3 production.

Local concentration of bacterial virulence factors has apparently a decisive effect on the metabolic state of the host cells in a given periodontitis site. Bacterial lipopolysaccharide has been shown to activate multiple MAP kinase in host cells (28, 29). *P. gingivalis* was found to elevate MMPs in human pulp (16) and periodontal ligament fibroblasts (17). However, no studies of the effect of *P. gingivalis* on gelatinases production have previously been reported in human osteosarcoma cells. In this study, we first found that MAPK inhibitors SB203580 and U0126 can inhibit *P. gingivalis*-induced MMP-9 activation.

In the last few years, the PI3K signal transduction pathway has emerged as one of the main signal routes that coordinate complex events lending to changes in cell metabolism, cell growth, cell movement, and cell survival (30). PI3K inhibitor was found to inhibit MMP-2 production in human breast cancer cells (31) and MMP-9 in ovarian carcinoma cell line OVCA 429 (32). Our data demonstrated that PI3K inhibitor down-regulated the MMP-9 in human osteosarcoma cells. In addition, PI3K inhibitor LY294002 was found to down-regulate the IL-1α and *P. gingivalis* up-regulated MMP-9 production. These results are in agreement with Esteve *et al.* (33), who found that PI3K inhibitor down-regulated MMP-9 expression induced by IL-1 in rat C6 glioma cells. However, our data were different from Kim and Koh (34), who reported that pretreatment with PI3K inhibitor lacked the ability to inhibit lipopolysaccharide-induced MMP activation in vascular endothelial cells. The difference between these contrary results is not clear; however, it may be a result of species variations in these experiments.

This study first demonstrated that human osteosarcoma cell line U2OS cells produce MMP-2 and MMP-9, and that MMP-9 production is enhanced by IL-1α and *P. gingivalis*, further supporting the notion that MMP-2 and MMP-9 are involved in the extracellular degradation during periodontitis (14). Furthermore, MMP-9 was inhibited by SB203580, U0126, and LY294002. These data indicate that pharmacological agents that target p38, MEK, and PI3K pathway in human osteosarcoma cells inhibit MMP-9 expression, and such inhibition may contribute to the pathogenesis of periodontal inflammation and that such inhibition might contribute to therapeutic efficacy. However, the detailed mechanism of activation of MMPs by bacterial infection as well as inflammatory cytokines *in vivo* remains to be further defined.

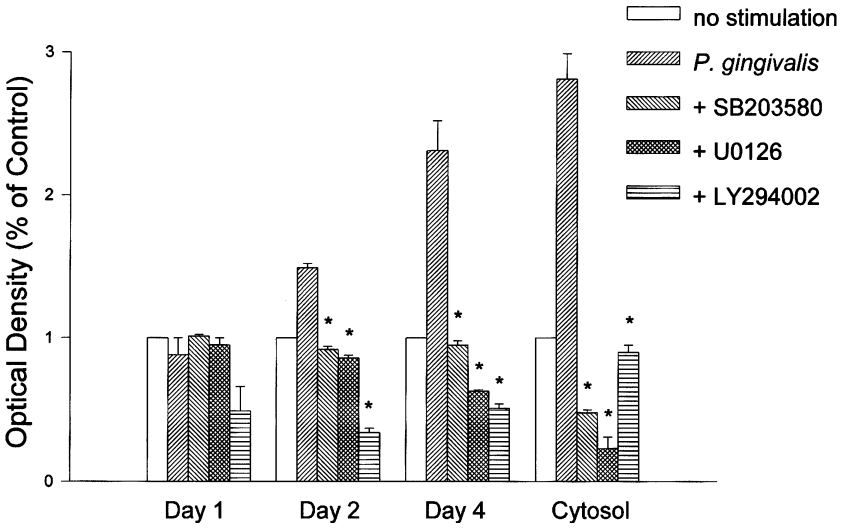

Fig. 4. Levels of matrix metalloproteinase-9 from conditioned medium and cytosol treated with *Porphyromonas gingivalis* in the presence of different kinase inhibitors were calculated from their gelatinolytic activity, as measured by AlphaImager 2000. Values are means and standard deviations of optical density from triplicate independent experiments. *Statistically significant between *P. gingivalis* alone and *P. gingivalis* added with different kinase inhibitors, $p < 0.05$. SB203580, p38 inhibitor; U0126, mitogen-activated protein kinase kinase (MEK) inhibitor; LY294002, phosphatidylinositaol 3-kinase (P13K) inhibitor.

Table 2. The effects of SB203580 (p38 inhibitor), U0126 [mitogen-activated protein kinase kinase (MEK) inhibitor], and LY294002 [phosphatidylinositaol 3-kinase (P13K) inhibitor] in the presence of *Porphyromonas gingivalis* on matrix metalloproteinase-9 activity, as measured by AlphaImager 2000. Values are means and standard deviations of optical density (percentage of control).

	P. gingivalis	+ SB203580	+ U0126	+ LY294002
Day 1	0.88 ± 0.12	1.01 ± 0.01[b]	0.95 ± 0.05[b]	0.49 ± 0.01[b]
Day 2	1.49 ± 0.03[a]	0.92 ± 0.02[b]	0.86 ± 0.02[b]	0.34 ± 0.03[b]
Day 4	2.31 ± 0.21[a]	0.95 ± 0.03[b]	0.63 ± 0.01[b]	0.51 ± 0.03[b]
Cytosol	2.81 ± 0.34[a]	0.48 ± 0.02[b]	0.23 ± 0.01[b]	0.90 ± 0.05[b]

[a]Statistically significant in comparison with control, $p < 0.05$.
[b]Statistically significant between interleukin-1α (IL-1α) alone and IL-1α + inhibitors, $p < 0.05$.

References

1. Woessner JF Jr. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J* 1991;**5:**2145–2154.
2. Birkedahl-Hansen H, Moore WGI, Bodden MK, *et al*. Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med* 1993;**4:** 197–250.
3. Gomez DE, Alonso DF, Yoshiji H, Thorgeirsson UP. Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. *Eur J Cell Biol* 1997;**74:**111–122.
4. Woessner JF, Nagase H. Matrix metalloproteinase and TIMPs. *Protein profile*. Oxford: Oxford University Press, 2000:1–9.
5. Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: current concepts. *J Periodontol* 1992;**63:** 322–331.
6. Gence RJ, Zamben JJ, Christerssun LA. The origin of periodontal infection. *Adv Dent Res* 1998;**12:**245–259.
7. Birkedal-Hansen H. Role of matrix metalloproteinases in human periodontal diseases. *J Periodontol* 1993;**64:**474–484.
8. Ryan ME, Golub LM. Modulation of matrix metalloproteinases activities in periodontitis as a treatment strategy. *Periodontol 2000* 2001;**24:**226–238.
9. Listgarten MA. Nature of periodontal diseases: pathogenic mechanisms. *J Periodont Res* 1987;**22:**172–178.
10. Robertson PB, Lantz M, Marucha PT, Kornman KS, Trummel CL, Holt SC. Collagenolytic activity associated with *Bacteroides* species and *Actinobacillus actinomycetemcomitans*. *J Periodont Res* 1982;**17:**275–283.
11. Schwartz Z, Goultschin J, Dean DD, Boyan BD. Mechanisms of alveolar bone destruction in periodontitis. *Periodontol 2000* 1997;**14:**158–172.
12. Nair SP, Megji S, Wilson M, Reddi K, Whtie P, Henderson B. Bacterially induced bone destruction: mechanisms and misconceptions. *Infect Immun* 1996; **64:**2371–2380.
13. Ingman T, Sorsa T, Lindy O, Koski H, Konttinen YT. Multiple forms of gelatinase/type IV collagenase in saliva and gingival crevicular fluid of periodontitis patients. *J Clin Periodontol* 1994;**21:**26–31.
14. Soell M, Elkaim R, Tenenbeam H. Cathepsin C, matrix metalloproteinases, and their tissue inhibitors in gingival and gingival crevicular fluid from periodontitis affected patients. *J Dent Res* 2002;**81:** 174–178.
15. Ejeil AL, Igondjo-Tchen S, Ghomrasseni S, Pellat B, Godeau G, Gogly B. Expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in healthy and diseased human gingival. *J Periodontol* 2003;**74:**188–195.
16. Chang YC, Lai CC, Yang SF, Chan Y, Hsieh YS. Stimulation of matrix metalloproteinases by black-pigmented *Bacteroides* in human pulp and periodontal ligament cell cultures. *J Endod* 2002; **28:**90–93.
17. Chang YC, Yang SF, Lai CC, Liu JY, Hsieh YS. Regulation of matrix metalloproteinases production by cytokines, pharmacological agents, and periodontal pathogens in human periodontal ligament fibroblast cultures. *J Periodont Res* 2002; **37:**196–203.
18. Huang FM, Chou MY, Chang YC. Induction of cyclooxygenase-2 mRNA and protein expression by epoxy resin and zinc oxide-eugenol based root canal sealers in human osteoblastic cells. *Biomaterials* 2003;**24:**1869–1875.
19. Senior RM, Griffin GL, Fliszar CJ, Shapiro SD, Goldberg GI, Welgus HG. Humen 92- and 72-kilodalton type IV collagenase and elastases. *J Biol Chem* 1991;**266:**7870–7875.
20. O'Grady RL, Nethery A, Hunter N. A fluorescent screening assay for collagenase using collagen labeled with L-methoxy-2,4-diphenyl-3(2H)furanone. *Anal Biochem* 1984;**140:**490–494.
21. Shen V, Kohler G, Jeffrey JJ, Peck WA. Bone-resorbing agents promote and interferon-r inhibits bone cell collagenase production. *J Bone Miner Res* 1988;**3:** 657–666.
22. Panagakos FS, Kumar S. Modulation of proteases and their inhibitors in immortal human osteoblast-like cells by tumor

necrosis factor-alpha *in vitro*. *Inflammation* 1994;**18:**243–265.

23. Lorenzo JA, Pilbeam CC, Kalinowski JF, Hibbs MS. Production of both 92- and 72-kDa gelatinases by bone cells. *Matrix* 1992;**18:**243–265.
24. Simon C, Goepfert H, Boyd D. Inhibition of the p38 mitogen-activated protein kinase by SB 203580 blocks PMA-induced Mr 92000 type IV collagenase secretion and *in vitro* invasion. *Cancer Res* 1998; **58:**1135–1139.
25. Reunanen N, Westermarck J, Hakkinen L, *et al*. Enhancement of fibroblast collagenases (matrix metalloproteinase-1) gene expression by ceramide is mediated by extracellular signal-regulated and stress-activated protein kinase pathway. *J Biol Chem* 1998;**273:**5137–5145.
26. Barchowsky A, Frleta D, Vincenti MP. Integration of the NF-kappa B and mitogen-activated protein kinase/AP-1 pathways at the collagenase-1 promoter: divergence of IL-1 and TNF-dependent signal transduction in rabbit primary synovial fibroblasts. *Cytokine* 2000;**12:** 1469–1479.
27. Domeij H, Yucel-Lindberg T, Modeer T. Signal pathways involved in the production of MMP-1 and MMP-3 in human gingival fibroblasts. *Eur J Oral Sci* 2002;**110:**302–306.
28. Sanghera JS, Weinstein SL, Aluwalia M, Girrn J, Pelech SL. Activation of multiple proline-directed kinases by bacterial lipopolysaccharide in murine macrophages. *J Immunol* 1996;**156:**4457–4465.
29. van der Bruggen T, Nijenhuis S, van Raaij E, Verhoef J, van Asbeck BS. Lipopolysaccharide-induced tumor necrosis factor alphaproduction by human monocytes involves the raf-1/MEK1-MEK2/ERK1-ERK2 pathway. *Infect Immun* 1999; **67:**3824–3829.
30. Cantley LC. The phosphoinositide 3-kinase pathway. *Science* 2002;**296:**1655–1657.
31. Sugiura T, Berditcherski F. Function of α3β1-tetraspanim protein complex in tumor cell invasion. Evidence of the role of the complexes in production of matrix metalloproteinase 2 (MMP-2). *J Cell Biol* 1999;**146:**1375–1389.
32. Ellerbroek SM, Halbleib JM, Benaridez M, *et al*. Phosphatidylinositol 3-kinase activity in epidermal growth factor-stimulated matrix metalloproteinase-9 production and cell surface association. *Cancer Res* 2001;**61:**1855–1861.
33. Esteve PO, Robledo O, Potworowski EF, St-Pierre Y. Induced expression of MMP-9 in C6 glioma cells is inhibited by PDGF *via* PI3-kinase-dependent pathway. *Biochem Biophys Res Commun* 2002;**296:**864–869.
34. Kim H, Koh G. Lipopolysaccharide activates matrix metalloproteinases-2 in endothelial cells through an NF-kappa B-dependent pathway. *Biochem Biophys Res Commun* 2000;**269:**401–405.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.