The role of p38 MAP kinase on RANKL regulation in mouse periodontal ligament fibroblasts

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I. INTRODUCTION

Chronic production of cytokines mediates inflammatory diseases. Proinflammatrory cytokine production in periodontal disease, as well as other chronic inflammatory diseases, such as high-turnover osteoporosis, results in a net bone loss that ultimately negatively affects host function[1,2]. A complex cytokine network controlled by many cell types, dictates cellular response in bone resorption. Several mediators of bone resorption and remodeling have been identified in response to proinflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α, as well as other bone-resorptive agents[3,4]. Bone marrow stromal cells respond to these agents and secret cytokines, including IL-6, IL-11, and GM-CSF, which in turn activate receptor activator of NF-κB ligand (RANKL), and others that signal osteoclasts to increase their numbers or action by resorbing bone[4-6]. Understanding signal transduction pathways in the osteoblast therefore are critical for osteoclast function and differentiation. Molecular targets that control bone marrow stromal cell cytokine production include transcription factors, such as NF-κB, and mitogen-activated protein (MAP) kinases. In response to the appropriate stimuli, many MAP kinases are activated by phophorylation events[7]. In recent years, the MAP kinases system has been shown to mediate in part, the actions of proinflammatory cytokines[7-9]. While it is getting clear that MAP kinases are involved in cytokine gene regulation, the mechanisms behind mRNA regulation are not fully understood.

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Periodontal ligament (PDL) resides between the cementum of the roots of the teeth and the alveolar bone. In this location, periodontal ligament fibroblasts are uniquely situated to maintain the overall integrity of the PDL[10]. Periodontal ligament fibroblasts not only function as support cells for periodontal tissues but also produce various inflammatory mediators[11,12].

In periodontal tissue, periodontopathic bacteria and the host cells stimulate the immune response, and the periodontal disease advances. The cells of periodontal tissue secrete a variety of immunoregulatory cytokines and chemical mediators for bone resorption such as IL-1, TNF, IL-6, and RANKL[13-15]. Recent studies have demonstrated that periodontal ligament fibroblasts express RANKL and this cytokine is regulated by p38 MAP kinase[15].

Involvement of p38 MAP kinase in RANKL production by IL-1β-induced human bone mouse stromal cells and by IL-1β -induced rat periodontal ligament fibroblasts was demonstrated by SB203580[15,16]. Because the inhibitor's effect was described only at the level of protein production, the mechanism of the inhibition of IL-1β-induced RANKL production by SB203580 still remains unclear. In addition, the regulation of MAP kinase in periodontal ligament fibroblasts by IL-1β has not been completely elucidated.

Thus understanding MAP kinase-mediating signalling pathways is essential to aid in generating therapeutics capable of treating disease mediated by RANKL such as rheumatiod arthritis or periodontitis. Therefore, the purpose of this study is to investigate the involvement of p38 MAP kinases in IL-1β-induced RANKL expression in mouse periodontal ligament fibroblast.

II. MATERIALS & METHODS

1. Cell culture of mouse periodontal ligament (mPDL) fibroblasts

Mouse PDL fibroblasts were obtained from the PDL tissues of the molar teeth obtained from BALB B/c mice. The PDL tissues were scraped from the middle of one third of the root surface and transferred into 6-well plates. The outgrown cells from the explant were cultured in DMEM (Invitrogen, U.S.A), supplemented with 10% fetal bovine serum (Sigma, U.S.A), 100 U/ml penicilline, and 100 ㎍/㎖ streptomycin, in a humidified atmosphere of 5% CO_2 in air at 37℃. At confluence, the cells were passaged with trypsin-EDTA and cultured on tissue culture plates.

2. RANKL immunoassay

mPDL fibroblasts were grown to near confluency in 12-well dishes and pretreated for 1 hour with several MAP kinase inhibitors, SB203580 (10 ㎛), p38 MAPK inhibitor; SP600125 (10 ㎛), JNK inhibitor; PD98059 (10 ㎛), ERK inhibitor; or NF-kB inhibitor (20 ㎛), and then stimulated with

IL-1β for 48 hours. Cell culture supernatants were harvested and concentrated with centrifugal filter tubes (Amicon, U.S.A). The amount of soluble RANKL (sRANKL) released from mPDL fibroblasts into the cultured supernatant was measured using an enzyme immunoassay kit (R&D Systems, U.S.A). Absorbance was determined at 540 nm by ELISA plate reader (Microplate reader, U.S.A).

3. Gene expression assessed by reverse transcription and polymerase chain reaction (RT-PCR)

Cells were grown to near confluency in 6-well dishes, deinduced and then pretreated with MAPKs inhibitors, SB203580 (10 μM) ; p38 MAPK inhibitor, SP600125 (10 μM); JNK inhibitor, PD98059 (10 μM); ERK inhibitor or NF-kB inhibitor (20 μM) for 1 hour and then stimulated with IL- 1β (1 nM) for additional 14~16 hours.

After the treatment periods, total RNA was collected with Trizol® reagent (Invitrogen, U.S.A), according to the manufacturer's instructions. Briefly, approximately 1 ㎖ of Trizol® was added to each well for cell lysis and this cell lysate was homogenized by passing several times through a pipette. This solution was transferred to eppendorf tubes, incubated at room temperature for 15~30 min, and then 0.2 ㎖ of chloroform per ㎖ of Trizol® was added. After vigorously shaking the tubes and centrifugation at 10,000 xg for 15 min at 2~8℃, the aqueous phase was collected and transferred to a fresh tube. Total RNA was precipitated by adding isopropyl alcohol, washed in 75% ethanol and redissolved in RNAse-free water for storage at -70℃ until use.

Using spectrophotometry, 5 ㎍ of RNA was quantitated (read at an absorbance of 260 nm) and used for cDNA synthesis with oligo (dT) 12~18 primer by reverse transcriptase (RT) reactions. Approximately 2 ㎕ of RT product was used as a template for the polymerase chain reaction (PCR).

The PCR products were checked by 2% agarose gel electrophoresis, stained by ethidium bromide. Semi-quantitative PCR was performed. Semi-quantitative differences of MMP-13 expression were normalized by GAPDH.

4. mRNA stability experiments

To determine the relative role of p38 MAP Kinase on IL-1β-induced RANKL mRNA stability, the rate of RANKL mRNA decay was determined in the presence and absence of the p38 inhibitor, SB203580 (10 μM). mPDL fibroblasts were pre-treated with SB203580 for 60 minutes, then treated with IL-1β (1 nM) for 16~18 hours. Subsequently, actinomycin D (2 ㎍/㎖), DNA polymerase inhibitor, was added to prevent further transcription. Total RNA was harvested and analyzed by RT-PCR as described above after 0, 30, 60, and 90 minutes treated with actinomycin D.

5. Effect of protein synthesis inhibitor on RANKL mRNA expression

To explore further mechanism behind the RANKL repression in the presence of the p38 MAP kinase inhibitor, experiment was performed in the presence of the protein synthesis inhibitor cycloheximide (CHX, 2 ㎍/㎖). mPDL fibroblasts were grown to near confluency in 6-well dishes and pre-treated for 30 minutes with CHX, and then treated for 1 hour with several MAP kinase inhibitors, SB203580 (10 μM), p38 MAPK inhibitor; SP600125 (10 μM), JNK inhibitor; PD98059 (10 μM), ERK inhibitor; or NF-kB inhibitor (20 μM), and stimulated with IL-1β. Total RNA was harvested and analyzed by RT-PCR as described above after 16 hours treated with CHX.

6. Western blot Analysis

mPDL fibroblasts were exposed to SB203580 for 30 minutes and then stimulated with IL-1b (1 ng/ml) for 30 minutes. After the treatment, whole cell lysates were collected for Western blot analysis of both active (phosphorylated) and inactive (non- phosphorylated) MAP kinase. Western blotting was conducted as described in the literature. Briefly, after removal of media by aspiration, cells were washed with ice-cold PBS (pH 7.4), and 100 μl of SDS-sample buffer was added to each well with removal of cells by scraping the wells with a disposable cell scraper. The cell lysate was transferred to Eppendorf tubes, boiled at 100℃ for 5 minutes, placed on ice, sonicated for 10-15 seconds and stored at -20℃ until use.

Following sample preparation by boiling at 100℃ for 5 min and placing on ice, SDS-PAGE was performed using pre-stained molecular weight markers to verify electro-transfer and biotinylated protein markers to determine molecular weights. After running the gel, proteins were blotted to a nitro-cellulose membrane.

The immunoblotting protocol was performed as described by the supplier of the commercial antibody kits. The nitrocellulose membrane was blocked for non-specific binding, washed and incubated with p38 mouse anti-human antibodies (Cell Signaling, U.S.A) using gentle agitation overnight at 4℃. After three washes, the membrane was incubated with the secondary anti-mouse antibodies conjugated to Horseradish peroxidase (Cell Signaling, U.S.A) and anti-biotin HRP-conjugated antibody with gentle agitation for 1 hour at room temperature. After three consecutive washes, the membrane was incubated with the enzyme substrate for 1 minute, wrapped in plastic film and exposed to X-ray film for 2 minutes.

7. Statistical analysis

Three different experiments were performed and the values were expressed as means ± SD. The significance of differences was calculated with student t-test or analysis of var-

Table 1. Amplification Primer Sets and Conditions used in Polymerase Chain Reaction

	Primer sequence	bps
OPG		
Forward	5'-TGTAGAGAGGATAAAACGG-3'	492
Backward	5'-CTAGTTATAAGCAGCTTAT-3'	
GAPDH		
Forward	5'-CACCATGGAGAAGGCCGGGG-3'	418
Backward	5'-GACGGACACATTGGGGGTAG-3'	
RANKL		
Forward	5'-CAGCACTCACTGCTTTTATAGAATC	499
Backward	5'-AGCTGAAGATAGTCTGTAGGTACGCC-3'	

OPG, osteoprotegerin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RANKL, receptor activator of NF-κB ligand

iance (ANOVA) and DUNCAN multiple range test were done using SAS program.

III. RESULTS

1. Selective inhibition of soluble RANKL (sRANKL) production

To determine the role of MAP kinases on sRANKL production in mPDL fibroblasts, quantitative ELISA specific for mouse RANKL was used. The amount of sRANKL was measured by a competitive immunoassay for sRANKL following treatment of several MAP kinase inhibitors (SB203580, p38 MAPK inhibitor; SP600125, JNK inhibitor; PD98059, ERK inhibitor) or NF-kB inhibitor and then stimulated with IL-1β for 48 hours. As shown in Figure 1, IL-1β stimulated sRANKL production. SB203580 repressed sRANKL production ($p<0.01$) whereas, sRANKL production was not inhibited with SP600125, PD98059, and NF-kB inhibitor. sRANKL production was dose- dependently inhibited by SB203580 (Figure 2).

2. Selective inhibition of RANKL mRNA expression

RANKL mRNA expression was determined by semi-quantitive PCR. Figure 3 showed that RANKL mRNA expression was increased approximately five-fold with IL-1β, but IL-1β-induced RANKL mRNA expression was inhibited by SB203580 (1.5 -fold reduction). However, there were no significant changes in RANKL mRNA by SP600125, PD98059, and NF-kB inhibitor.

3. p38 was needed to mediate IL-1β-induced RANKL mRNA stability.

Actinomycin D was used to determine if p38 MAP Kinase regulated IL-1β-induced RANKL mRNA stability. The results in-

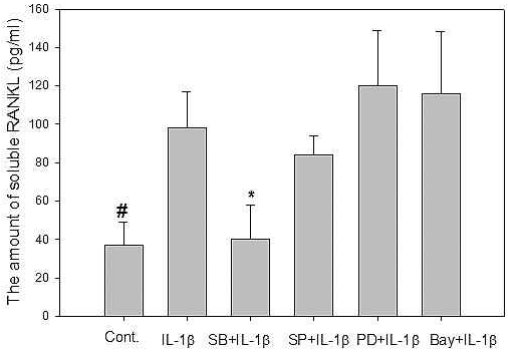

Figure 1. p38 MAP kinase is needed for IL-1β -induced sRANKL production in mPDL fibroblasts. Cells were treated with MAP kinase inhibitors (SB203580, p38 MAPK inhibitor; SP600125, JNK inhibitor; PD98059, ERK inhibitor) or NF-kB inhibitor and then stimulated with IL-1β for 48 hours. The statistical significance of difference was evaluated by ANOVA (*p<0.01). #P values compare control to IL-1β-stimulated sample. *P values compare p38 MAP kinase inhibitor to other inhibitors.

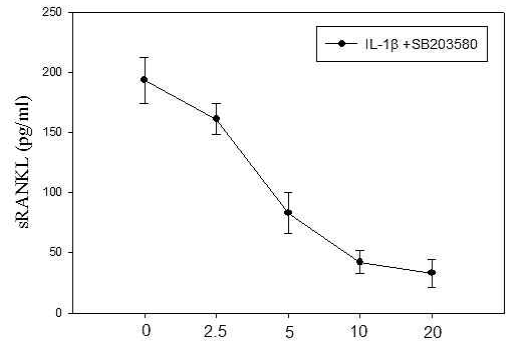

Figure 2. SB203580 inhibits sRANKL secretion in a dose-dependent manner in mPDL fibroblasts. mPDL fibroblasts were pretreated with SB203580 at indicated concentrations for 1 hour and then stimulated with IL-1β (1 ng/ml) for 48 hours.

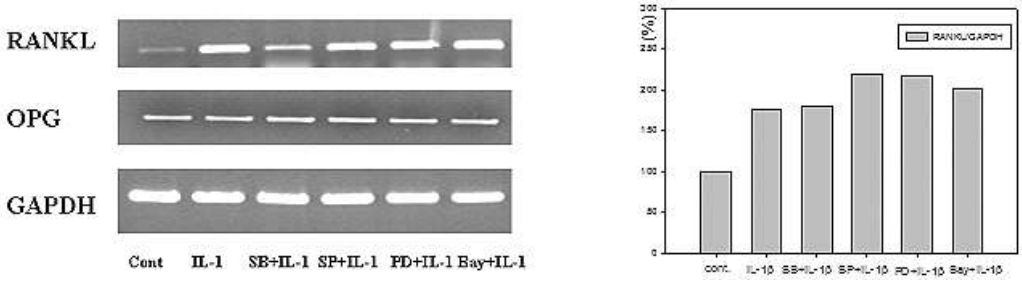

Figure 3. RT-PCR analysis of receptor activator of NF-κB ligand (RANKL) and glyceraldehyde -3-phosphate dehydrogenase (GAPDH) mRNA expression in mPDL fibroblasts. RANKL mRNA expression is required by p38 MAP kinase when stimulated by IL-1β (1 ng/ml).

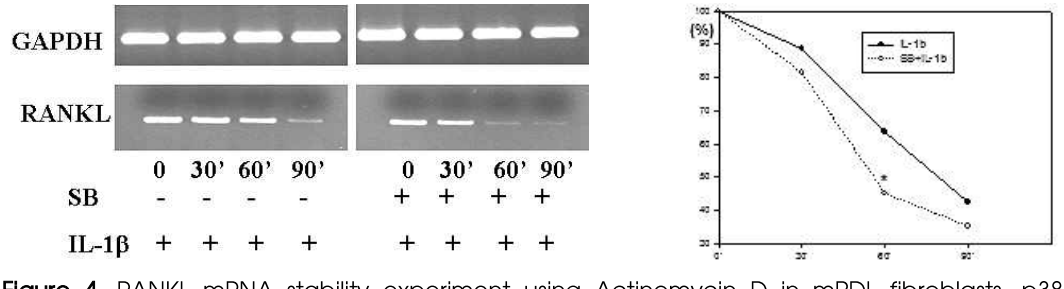

Figure 4. RANKL mRNA stability experiment using Actinomycin D in mPDL fibroblasts. p38 MAP kinase mediates IL-1β-induced RANKL mRNA stability.
* indicates half lifes of RANKL mRNA in the presence of SB203580.

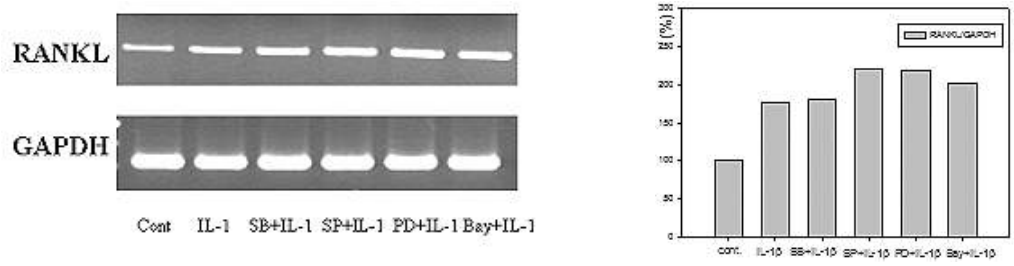

Figure 5. Effect of protein synthesis inhibitor on RANKL mRNA expression. De novo protein synthesis is required to mediate RANKL expression. mPDL fibroblasts were treated with IL-1β (1 ng/ml) and SB203580 (10 μM) and in the presence or absence of cycloheximide (2 ㎍/㎖). Cycloheimide blocked the ability of SB203580 to mediate its effect.

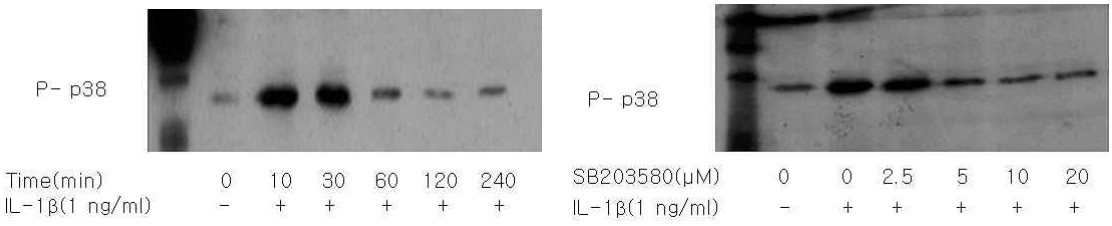

Figure 6A. p38 MAP kinase phosphorylation is increased for 30 minutes with IL-1β (1 ng/ml) then decreased for 2 hours, **B.** p38 MAP kinase phosphorylation is dose-dependently inhibited by SB203580 in mPDL fibroblasts. Cells were treated with SB203580 at indicated concentrations for 60 minutes and then stimulated for 30 minutes with IL-1β (1 ng/ml).

dicate that IL-1β increased the half-life of RANKL, with 90 minutes, but in the presence of SB203580, the half life was decreased to 60 minutes following normalization to GAPDH mRNA (Figure 4).

4. De novo protein synthesis was required to mediate RANKL mRNA expression

To investigate further the mechanism of the RANKL mRNA repression in the presence of SB203580, experiments were performed in the presence of the protein synthesis inhibitor cycloheximide (CHX). mPDL fibroblasts were treated with IL-1β (1 ng/ml) and SB203580 (10 μM) and in the presence or absence of CHX (2 ㎍/㎖). As shown in Figure 5, CHX blocked the ability of SB203580 to mediate its effect.

5. Activation of p38 MAP kinase was inhibited with treatment with SB203580

In the time course study, p38 MAP kinase phosphorylation was increased for 30 minutes with IL-1β (1 ng/ml) then decreased for 2 hours. Western blot analysis of antibodies indicated that phosphorylation of p38 MAP kinase was activated by IL-1β in mPDL fibroblasts, stimulated for 30 minutes. SB203580 above 2.5 mM, was able to block IL-1β-induced p38 MAP kinase.

IV. DISCUSSION

Bone is a dynamic tissue that constantly undergoes a remodeling process where bone resorption and bone deposition are balanced. When chronic inflammation occurs in bone, this balance is disrupted, thus favoring net bone loss. Diseases involving an intimate combination of bone loss and inflammation include periodontal disease and rheumatoid arthritis or metabolic bone diseases such as osteoporosis, Paget's disease, and multiple myeloma in which cytokine deregulation is observed.

Bone resorptive agents, such as parathyroid hormone (PTH), TNF-α, and IL-1β have all shown to stimulate RANKL production and secretion in osteoblasts[1-3]. Proinflammatory cytokines, such as IL-1β and TNF-α, involved in bone formation and remodeling, converge on the expression of receptor-activated NF-κB ligand (RANKL), and its decoy receptor, osteoprotegerin (OPG). RANKL and OPG expression from stromal/osteoblastic cells increases osteoclastogenesis through activation of the RANKL cognate receptor, RANK, located on osteoclast precursor cells[15-17].

MAP kinases are key enzymes in the signal transduction cascade from the extracellular environment to the nucleus of every essential eukaryotic cell type. Three groups of MAP kinases have been identified in mammalian cells. These are the ERK, the JNK, and p38 MAP kinase which are activated by environmental stress and inflammatory cytokines[18]. p38 MAP kinase was originally identified as the target of pyridinylimidazole compounds that inhibit the production of inflammatory cytokines from monocytes. Subsequently, it has been shown to play a role in a variety of other cellular process, including prolongation of cytokine mRNA half-life[7,9].

RANKL and osteoprotegerin (OPG) have been shown to play very important roles in bone remodeling. RANKL, which is a type II transmembrane protein, consists of a receptor-binding ectodomain and a membrane-anchored domain[19,20]. The membrane-bound RANKL is cleaved to a soluble RANKL by the metalloproteinase-disintegrin TNF-α convertase or a related metalloproteinase[21]. RANKL, which is produced by osteoblasts and bone marrow stromal cells, activates mature osteoclasts during growth and osteoclastogenesis via their receptor activator of nuclear kB (RANK) in pre-osteoclasts[17].

PDL fibroblasts also play a role in osteoclastogenesis through RANKL expression on their cell surfaces[22-24]. Understanding how membrane-bound RANKL and souble RANKL are regulated at the level of expression and function is important in elucidating the role of RANKL on osteoclastogenesis in alveolar bone. However, in previous studies, the role of RANKL in osteoclastogenesis has been demonstrated in osteoblasts and bone marrow stromal cells[25,26]. Inhibition of RANKL expression or control of RANKL production in PDL fibroblasts would repress osteoclasto-

genesis and present an attractive target for the prevention and the treatment of periodontitis. Recent studies have demonstrated that IL-1β-induced RANKL production can be blocked with p38 MAP kinase inhibitors in bone marrow stromal cells. However, transduction pathways in RANKL expression have not been completely understood. Therefore, this study was done to elucidate the role of MAP kinases on RANKL expression and production in mouse PDL fibroblasts.

A soluble RANKL has been shown to activate osteoclasts via RANK on osteoclasts[17]. In the present study, stimulation with IL-1β was shown to increase sRANKL and decrease sRANKL production by SB203580, when measured by ELISA. It means that sRANKL protein production is regulated by p38 MAP kinase.

In this study, it was determined the relative role of p38 MAP kinase in IL-1β -induced RANKL expression. Several intracellular signaling protein inhibitors were used to explore the possible molecular events that were involved RANKL expression. Because RANKL gene expression is one of the major regulators of osteoclast activation, we wanted to determine which signaling pathway was involved in RANKL expression. Figure 4 showed that IL-1β stimulated RANKL mRNA by 5 folds. However, SB203580, p38 MAP kinase inhibitor, was able to reduce IL-1β-induced RANKL gene expression. No agents used in this study affected OPG gene expression. This result indicated that p38 MAP kinase mediated RANKL expression whereas, it did not regulate OPG expression in mPDL fibroblasts.

The mechanism of p38 regulation of RANKL mRNA expression could occur at the transcriptional or post-transcriptional level. To determine whether p38 stabilized RANKL mRNA by post-transcriptional regulation, mRNA degradation studies were performed. SB203580 altered IL-1β-induced RANKL mRNA decay rates in PDL fibroblasts which indicated that in PDL fibroblasts, p38 MAP kinase would mediated its effects on enhancing RANKL gene expression mainly through the stabilization of RANKL mRNA. As shown in Figure 4, p38 MAP kinase mediates IL-1β-induced RANKL mRNA stability. However, SB203580 reduced the half life of the RANKL from approximately 90 minutes to less than 60 minutes in IL-1β-stimulated cells in the presence of Actinomycin D, an inhibitor of DNA-primed RNA polymerase, to arrest transcription. These results indicated that p38-induced RANKL expression involved post-transcriptional stability.

The cytokines, IL-1β and RANKL have been reported to induce bone resorption[16,25]. It has also been shown that the tyrosine kinase inhibitor inhibits bone resorption[26] suggesting that tyrosine kinase may also be involved in the process of bone resorption. Furthermore, it is noteworthy that the level of IL-1β as well as IL-6 is enhanced in crevicular fluids from patients with gingival inflammation[27]. However, in the present study,

herbimycin, a known tyrosin kinases, did not inhibit IL-1β-induced RANKL mRNA expression in mPDL fibroblasts (data unshown). So, inhibition of p38 MAP kinase can block RANKL mRNA expression.

To explore further the mechanism of the RANKL mRNA expression in the presence of SB203580, another experiment was done in the presence of the protein synthesis inhibitor cycloheximide (CHX). As shown in Figure 5, CHX blocked the ability of SB203580 to mediate its effect. This result indicated that de novo protein synthesis (=onging protein synthesis) was required for RANKL mRNA expression.

Many hormones and cytokines regulate stromal cell and osteoblastic cell-derived RANKL expression. Inflammatory mediators, including IL-1β and TNF-α have been shown to mediate their bone resorptive properties through RANKL induction in stromal, osteoblasts, and periodontal ligament fibroblasts[15,16,28,29]. In these studies, signaling mechanisms involved in IL-1β and TNF-α -induced RANKL expression indicated that p38 MAP kinase was a critical component[9,15,16]. The present results were in line with previous studies that IL-1β-induced RANKL expression required p38 activation in mouse PDL fibroblasts. And pre-treatment with SB203580 decreased RANKL mRNA expression. Therefore, these results support the role of p38 MAP kinase inhibitors to reduce RANKL production and would be provide therapeutic targets in the management of inflammatory bone diseases such as periodontitis and rheumatoid arthritis.

In summary, this study has been demonstrated that p38 MAP kinase is a crucial signaling intermediate in RANKL mRNA expression in mouse periodontal ligament fibroblasts. A major point of p38-dependent RANKL regulation occurs at the level of mRNA stability. To understand exact signalling mechanism which contributes towards RANKL expression, further studies are needed at transcriptional and post-transcriptional level.

V. CONCLUSION

Several mediators of bone resorption have been identified in response of proinflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor-α Bone marrow stromal cells and osteoblasts respond to these cytokines that stimulate the production of receptor activation of nuclear factor κB ligand (RANKL).

Periodontal ligament (PDL) fibroblasts also play a role in osteoclastogenesis through RANKL expression on their cell surface. Understanding RANKL regulation in a gene level is important in elucidating the role of RANKL on osteoclastogenesis in alveolar bone. Recent studies have demonstrated that IL-1β-induced RANKL production can be blocked with p38 MAP kinase inhibitors in rat PDL fibroblasts. However, transduction pathways in RANKL expression have not been fully understood. Therefore, this study was done to elucidate the role of MAP kinases on RANKL regulation in mouse PDL fibroblasts.

The results were as follows;

1. IL-1β stimulated soluble RANKL (sRANKL) production in mPDL fibroblasts. sRANKL production was inhibited in a dose-dependent manner by SB203580, p38 MAP kinase inhibitor when stimulated with IL-1β ($p<0.01$). However, RANKL production was not inhibited with SP600125, PD98059, and NF-kB inhibitor.
2. RANKL gene expression was increased approximately five-fold with IL-1β, but IL-1β-induced RANKL expression was inhibited by SB203580 (1.5-fold reduction). However, there were no significant changes in RANKL genes by SP600125, PD98059, and NF-kB inhibitor.
3. IL-1β increased the half-life of RANKL, with 90 minutes, but in the presence of SB203580, the half life was decreased to 60 minutes following normalization to GAPDH.
4. Inhibitory effects of RANKL mRNA required de novo protein synthesis.
5. In western blot analysis, activation of p38 MAP kinase was inhibited with treatment with SB203580.

These results suggest that p38 MAP kinase regulate IL-1β-stimulated RANKL gene expression in mouse periodontal ligament fibroblasts.

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- 국문초록 -

마우스 치주인대 섬유모세포에서 RANKL 조절에 대한 p38 MAP kinase의 역할

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Receptor activation of nuclear factor κB ligand (RANKL)은 파골세포의 분화와 기능에 중요한 역할을 하는 단백질로 이들 물질의 조절에는 p38 MAP kinase가 관여한다. 그러나 치주인대 섬유모세포에서 RANKL 발현 시 p38 MAP kinase의 역할은 잘 알려져 있지 않다. 이에 이번 연구는 마우스 치주인대 섬유모세포의 IL-1β-induced RANKL 발현과정에서 p38의 역할을 규명하고자 하여 다음과 같은 결과를 얻었다.

마우스 치주인대 섬유모세포에 IL-1β (1 ng/㎖)의 자극은 수용성 RANKL의 합성을 증가시켰다. 수용성 RANKL의 합성은 p38 MAP kinase 억제제인 SB203580에 의해 농도 의존적으로 억제되었으나 다른 MAP kinase 억제제인 SP600125, JNK 억제제와 PD98059, ERK 억제제에 의해서는 수용성 RANKL의 합성이 조절되지 않았다. NF-kB 억제제에 의해서도 수용성 RANKL의 합성이 억제되지 않았다. RANKL 유전자의 발현은 IL-1β로 자극 시에는 대조군에 비해 약 5배의 발현 증가를 보였으나 SB203580으로 전처치 시 IL-1β (1 ng/㎖)로 자극시보다 약 1.5배의 감소를 보였다. 그러나 SP600125, PD98059, 및 NF-kB 억제제로 전처치한 경우에는 IL-1β로 자극한 경우와 비슷한 수준을 보였다. IL-1β로 자극 시 RANKL 유전자의 반감기가 90분 이었으나 SB203580로 전처치 후 IL-1β로 자극 시 RANKL 유전자의 반감기는 60분으로 감소하였다. Cycloheximide 전처리 시 SB203580에 의한 RANKL 유전자 발현 억제가 관찰되지 않았다. 단백질 분석결과 p38 MAP kinase의 인산화 활성은 30분까지 증가하였으나 그 이후 감소하여 2시간째에는 그 발현이 미약하였다. SB203580로 전처치 후 IL-1β로 자극 시 p38 MAP kinase의 인산화 활성이 감소하였다.

이상의 결과는 p38 MAP kinase 가 RANKL 유전자 조절에 중요한 역할을 담당하고 있음을 시사한다.

Key worlds: RANKL, p38 MAP kinase, ELISA, PCR