Gomisin N decreases inflammatory cytokine production in human periodontal ligament cells

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Text pages 16 (including 1 title pages), 3 Figures,

Running head; Gomisin N inhibits inflammatory cytokine production.

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Keywords: gomisin N, inflammatory cytokine, MAPKs, periodontal ligament cells

Abstract

Gomisin N, which is a lignan isolated from Schisandra chinensis, has some pharmacological effects. However, the anti-inflammatory effects of gomisin N on periodontal disease are uncertain. The aim of this study was to examine the effect of gomisin N on inflammatory mediator production in tumor necrosis factor (TNF)-α-stimulated human periodontal ligament cells (HPDLC). Gomisin N inhibited interleukin (IL)-6, IL-8, CC chemokine ligand (CCL) 2, and CCL20 production in TNF-α-stimulated HPDLC in a dose-dependent manner. Moreover, we revealed that gomisin N could suppress extracellular signal-regulated kinase (ERK) and c-Jun N terminal kinase (JNK) phosphorylation in TNF-α-stimulated HPDLC though protein kinase B (Akt) phosphorylation was not suppressed by gomisin N treatment. In summary, gomisin N might exert antiinflammatory effects by attenuating cytokine production in periodontal ligament cells via inhibiting the TNF-α-stimulated ERK and JNK pathways activation.

Introduction

Gomisin N is a bioactive compound isolated from Schisandra chinensis, which is used for traditional chinese medicine to treat diabetes, asthma, and respiratory infection. Gomisin N exerts a variety of pharmacological effects, including anti-inflammation [1] and anti-cancer effect [2]. Previous study showed that gomisin N suppresses PMA-induced inflammatory response in bone marrow-derived mast cells by inhibiting IL-6, prostaglandin D2, leukotriene C4, and cyclooxygenase-2 [3]. Another report showed that gomisin N enhances tumor necrosis factor α-induced apoptosis in HeLa cell [4]. This result showed that gomisin N might be use for an anti-cancer drug. However, it is uncertain whether gomisin N has anti-inflammatory effects on periodontal resident cells.

Periodontal disease is an infectious inflammatory disease induced by periodontal pathogenic bacteria. Host response to the bacteria is essential for the initiation and progression of periodontal disease [5]. Inflammatory cytokines from periodontal resident cells are important to explain the pathogenesis of periodontitis [5]. Interleukin (IL)-6 is involved in the activation of osteoclast in periodontal lesion [6]. Chemokines, such as IL-8, CCL2, and CCL20, induce the migration and accumulation of leukocytes, including neutrophils [7], macrophages [8], and Th17 cells [9]. Therefore, the inhibition of too much cytokine production could lead the relief of inflammation in periodontal lesion.

The aim of this study was to examine the effect of gomisin N on IL-6, IL-8, CCL2, and CCL20 production from TNF-α-stimulated human periodontal ligament cells (HPDLC)

Moreover, we examined if gomisin N treatment modified the activation of p38 mitogen activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), and c-Jun N terminal kinase (JNK), and protein kinase B (Akt) pathway in TNF-α-stimulated HPDLCs.

Materials and Methods

Cell culture.

Human primary periodontal ligament cells were obtained from Lonza Japan Ltd (Chuo-ku, Tokyo, Japan) and grown in the Dulbecco's modified Eagle's medium (DMEM: Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS), 1 mmol/L sodium pyruvate (Gibco) and, antibiotics (penicillin G: 100 units/ml, streptomycin: 100μg/ml: Gibco) at 37°C in a humidified air with 5% CO_2. HPDLC were seeded in 24-well plates at a density of 1×10^5 cells per well for ELISA or in 12-well plates at a density of 3×10^5 per well for western blot analysis. We used HPDLC for our experiments after confluence. Cells were used between passage numbers 5 and 10.

IL-6, IL-8, CCL2, and CCL20 production in HPDLC.

The HPDLC were stimulated with recombinant human TNF-α (10 ng/ml: Peprotech, Rocky Hill, NJ, USA) for 24 hours. The supernatants from the HPDLC were collected, and the IL-6, IL-8, CCL2, and CCL20 concentrations in the culture supernatants were measured in triplicate with ELISA. Duoset (R&D systems, Minneapolis, MN, USA) was used for the determination. All assays were performed according to the manufacturer's instructions, and cytokine levels were determined using the standard curve prepared for each assay. In selected

experiments, the HPDLC were cultured for 1 hour in the presence or absence of gomisin N (3.125, 6.25, 12.5, 25, or 50 μM: Nagara Science Co., Ltd., Gifu, Japan), SB203580 (p38 MAPK inhibitor, 10 μM: Santa Cruz Biotechnology, Santa Cruz, CA, USA), PD98059 (ERK inhibitor, 10 μM: Cayman Chemical, Ann Arbor, MI, USA), SP600125 (JNK inhibitor, 10 μM: Enzo Life Sciences, Plymouth Meeting, PA, USA), or 10-DEBC hydrochloride (Akt inhibitor, 1 μM: Santa Cruz Biotechnology) prior to the incubation with TNF-α (10 ng/ml).

Western blot analysis

To detect the TNF-α-induced phosphorylation of signal transduction molecules, western blot analysis was performed. HPDLC stimulated by TNF-α (10 ng/ml) with or without gomisin N (12.5 μM or 25 μM) pretreatment for 1 hour were washed once with cold PBS, before being incubated on ice for 30 min with cell lysis buffer (Cell signaling technology, Danvers, MA, USA) supplemented with protease inhibitors cocktail (Sigma). After removal of debris by centrifugation, the protein concentrations of the lysates were quantified with the Bradford protein assay using IgG as a standard. A 20 μg protein sample was loaded onto a 4-20% SDS-PAGE gel, before being electrotransfered to a PVDF membrane. The phosphorylation of p38 MAPK, ERK, JNK, and Akt was assessed using phospho-p38 MAPK rabbit monoclonal antibody (Cell Signaling Technology), phospho-ERK rabbit monoclonal antibody (Cell Signaling Technology), phospho-JNK rabbit monoclonal antibody (Cell Signaling Technology), phospho-Akt rabbit monoclonal antibody (Cell Signaling Technology), p38 MAPK rabbit monoclonal antibody (Cell Signaling Technology), ERK

rabbit monoclonal antibody (Cell Signaling Technology), JNK rabbit monoclonal antibody (Cell Signaling Technology), Akt mouse monoclonal antibody (Cell Signaling Technology), or glyceraldehyde-3-phosphate dehydrogenasee (GAPDH) rabbit monoclonal antibody (Cell Signaling Technology) according to the manufacturer's instructions. Protein bands were visualized by incubation with the HRP-conjugated secondary antibody (Sigma), followed by detection using the ECL Prime Western Blotting Detection System (GE Healthcare, Uppsala, Sweden). Each chemiluminescent signal was quantified by the ImageJ software (version 1.44).

Statistical analysis.

Statistical significance was analyzed using the Student's *t* test. *P* values<0.05 were considered significant in Fig.1 and 3.

Results

Effects of gomisin N on IL-6, IL-8, CCL2, and CCL20 production in TNF-α-stimulated HPDLC.

To examine the anti-inflammatory effects on gomisin N, IL-6, IL-8, CCL2, and CCL20 productions were measured using ELISA. Fig.1 shows that TNF-α stimulation induced IL-6, IL-8, CCL2, and CCL20 in HPDLCs. Treatment of gomisin N significantly suppressed IL-6, IL-8, CCL2, and CCL20 productions in a dose-dependent fashion (Fig.1) Method of transcriptional and translational (MTT) assays revealed treatment with 25 or 50 μM of gomisin N for 24 h did not modulate HPDLC viability (data not shown).

Effects of gomisin N on MAPKs and Akt activations in TNF-α-stimulated HPDLC

MAPKs and Akt signaling pathways play essential roles in the regulation of cytokine and chemokine productions in some types of cells [10, 11]. Therefore, we investigated if the anti-inflammatory effects of gomisin N resulted from the inhibition of MAPKs and Akt pathways. Fig.2 shows that gomisin N (25 μM) treatment suppressed ERK and JNK phosphorylation in TNF-α-stimulated HPDLC.

Effects of MAPKs and Akt inhibitors on IL-6, IL-8, CCL2, and CCL20 productions in TNF-α-stimulated HPDLC.

Finally, we investigated which signaling pathways are related with IL-6, IL-8, CCL2, and CCL20 production in TNF-α-stimulated HPDLCs using chemical inhibitors. A p38 inhibitor (SB203580) and an ERK inhibitor (PD98059) significantly suppressed IL-6, IL-8, CCL2, and CCL20 productions in TNF-α-stimulated HPDLC. A JNK inhibitor (SP600125) inhibited IL-6, IL-8, and CCL2 productions though CCL20 production was not changed by a JNK inhibitor (SP600125) treatment. An Akt inhibitor significantly decreased IL-8 and CCL2 productions though IL-6 and CCL20 productions from TNF-α-stimulated HPDLC were not modified.

Discussion

Immune response to the bacteria is important for the initiation and progression of periodontal disease [5]. Inflammatory cytokines are related to bone resorption in periodontal lesion [12]. Leukocytes, such as T cells and macrophages, are involved in the pathogenesis of periodontal

disease [13], and chemokines are essential for the accumulation of leukocytes in inflammatory lesion [14]. Therefore, the inhibition of cytokine and chemokines in periodontal lesion is important for the treatment of periodontal disease. In this study, gomisin N could decrease IL-6, IL-8, CCL2, and CCL20 productions in HPDLC which is a resident cell in periodontal tissue. IL-6 is related to the activation and differentiation of osteoclasts in periodontal lesion [6]. IL-8 is able to induce neutrophils accumulation [7]. It is certain that neutrophils could induce bone resorption in periodontal diseased tissue [14]. CCL2 and CCL20 are chemokines that are related to macrophages and Th17 cells migration and accumulation [9, 10]. It is certain that Th17 cells are important T cell subsets to explain bone destruction in inflammatory disease, such as arthritis [9] or periodontitis [16]. In this study, gomisin N significantly suppressed IL-6, IL-8, CCL2, and CCL20 productions from TNF-α-stimulated HPDLC. This result means that gomisin N might protect bone destruction in periodontal lesion. Further investigation using animal model is necessary to address this hypothesis.

In this study, we showed gomisin N could inhibit MAPKs activations in TNF-α-stimulated HPDLC. Previous manuscripts reported the effect of gomisin N on MAPKs phosphorylation. Waiwut and the colleagues reported that gomisin N could suppress p38 MAPK phosphorylation in TNF-α-treated Hela cells [4]. Park and the colleagues reported that gomisin N inhibited p38 MAPK and JNK phosphorylation in toll-like receptor (TLR) 2 and 4 agonists stimulated microglia [17]. They also showed that gomisin N did not modulate ERK

activation in TLR 2/4 agonists treated microglia [17]. We revealed gomisin N suppressed ERK and JNK phosphorylation in TNF-α-treated HPDLC. We think the effect of gomisin N on MAPKs activation is dependent on the type of cells and stimulants.

In summary, this report shows that gomisin N could inhibit inflammatory mediators, including IL-6, IL-8, CCL2, and CCL20, productions in HPDLC through inhibition of TNF-α-induced ERK and JNK activation. Further study should be necessary to address the question whether we can use gomisin N for the treatment of periodontal disease.

Acknowledgements

This work was supported by JSPS KAKENHI (Grant Numbers: 16K11834 and 15K11392).

Conflict of interest

The authors confirm that they are no conflicts of interest.

Figure Legends

Fig.1. Effects of gomisin N on IL-6, IL-8, CCL2, and CCL20 production from TNF-α-stimulated HPDLC. HPDLC were pretreated with gomisin N (3.125, 6.25, 12.5, 25, or 50 μM) for 1 hour, and then the HPDLC were stimulated with TNF-α (10 ng/ml), and the supernatants were collected after 24 hours. The expression levels of IL-6, IL-8, CCL2, and CCL20 in the supernatants were measured using ELISA. The results are shown as the mean and SD of one representative experiment performed in triplicate. The error bars show the SD of the values. * = P<0.05, significantly different from the TNF-α stimulated HPDLC without gomisin N.

Fig.2. Effects of gomisin N on p38 MAPK, ERK, JNK, and Akt phosphorylation in TNF-α-stimulated HPDLC. HPDLC were pretreated with gomisin N (12.5 or 25 μM) for 1 hour, and then the HPDLC were stimulated with TNF-α (10 ng/ml), and proteins were collected after 15, 30, or 60 minutes. Phospho-p38 MAPK, total-p38 MAPK, phospho-ERK, total-ERK, phospho-JNK, total-JNK, phospho-Akt, and total-Akt were determined by western blot analysis. We show a representative data from three independent experiments.

Fig.3. Effects of MAPK inhibitors and Akt inhibitor on IL-6, IL-8, CCL2, and CCL20 production from TNF-α-stimulated HPDLC. HPDLC were pretreated with SB203580 (p38 MAPK inhibitor, 10 μM), PD98059 (ERK inhibitor, 10 μM), SP600125 (JNK inhibitor, 10 μM), or 10-DEBC hydrochloride (Akt inhibitor, 1 μM) for 1 hour, and then HPDLC were stimulated with TNF-α (10 ng/ml), and the supernatants were collected after 24 hours. The production levels of IL-6, IL-8, CCL2, and CCL20 in the supernatants were measured using ELISA. The results are shown as the mean and SD of one representative experiment performed in triplicate. The error bars show the SD of the values. * = $P<0.05$, significantly different from the TNF-α stimulated HPDLC without chemical inhibitors.

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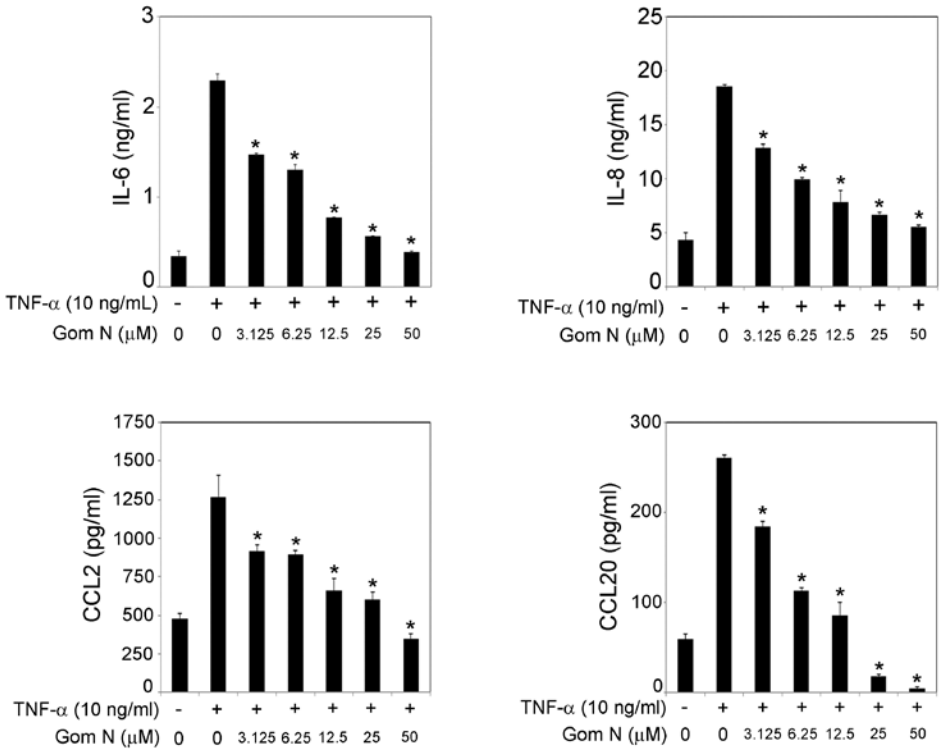
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IL-8 (ng/ml)
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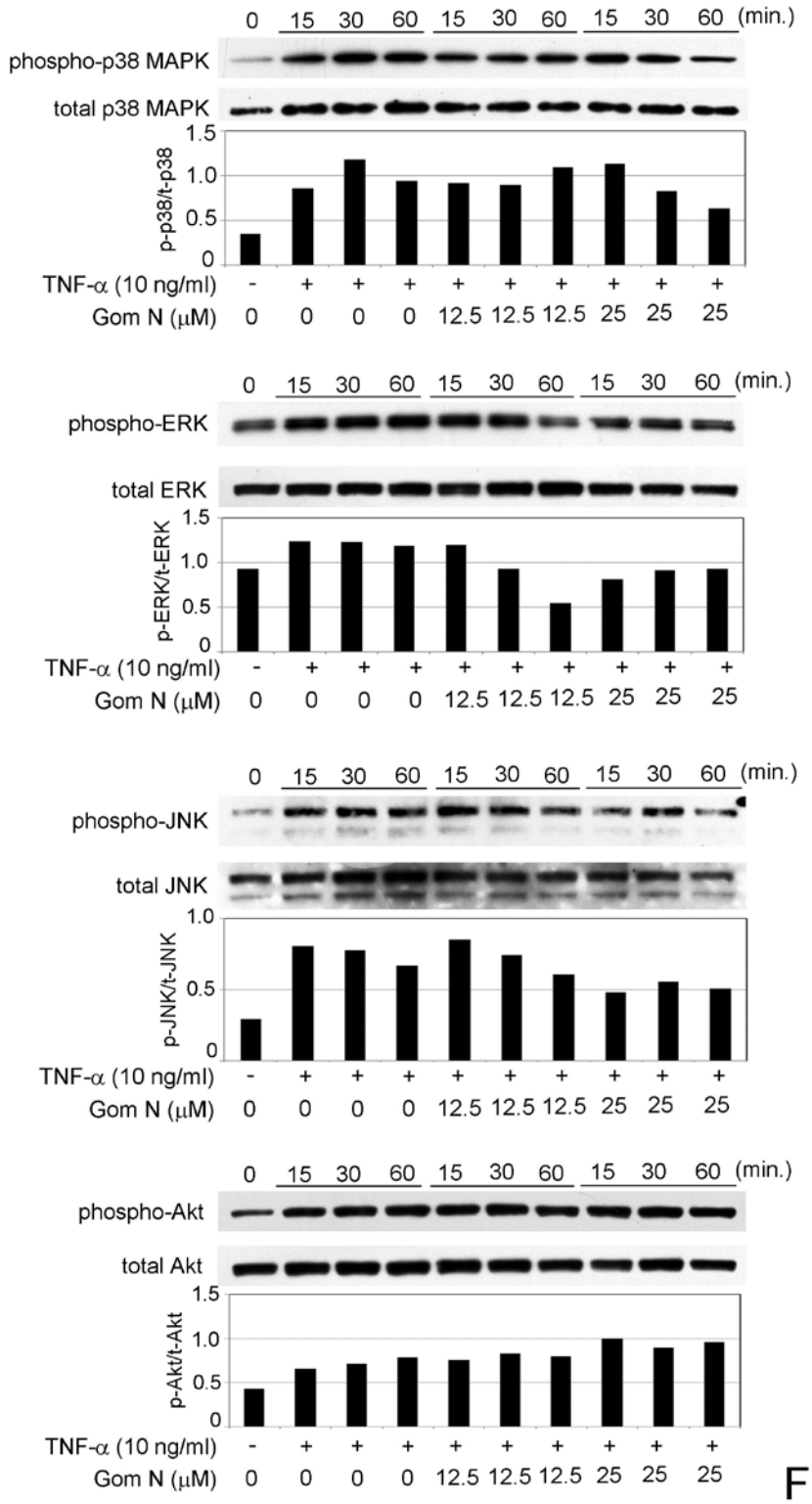
Fig. 1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phospho-ERK
total ERK
p-ERK/t-ERK
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TNF-α (10 ng/ml) - + + + + + + + + +
Gom N (μM) 0 0 0 0 12.5 12.5 12.5 25 25 25
0 15 30 60 15 30 60 15 30 60 (min.)
phospho-JNK
total JNK
p-JNK/t-JNK
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phospho-Akt
total Akt
p-Akt/t-Akt
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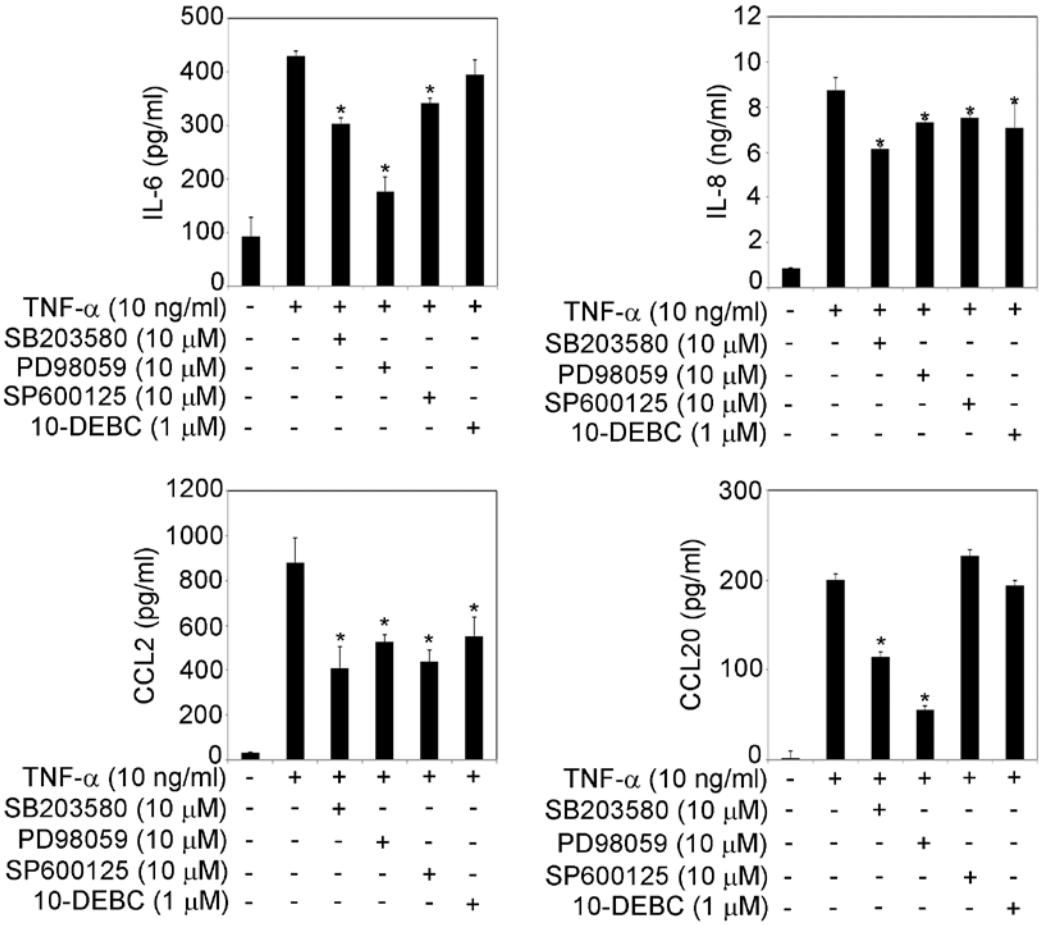
Fig. 2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SP600125 (10 μM) - - - - + -
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Fig. 3