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Effects of Methyl Gallate and Gallic Acid on the Production of Inflammatory Mediators Interleukin-6 and Interleukin-8 by Oral Epithelial Cells Stimulated with *Fusobacterium nucleatum*

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Interactions between periodontal bacteria and human oral epithelial cells can lead to the activation and expression of a variety of inflammatory mediators in epithelial cells. *Fusobacterium nucleatum* is a filamentous human pathogen that is strongly associated with periodontal diseases. This study examined the effects of methyl gallate (MG) and gallic acid (GA) on the production of inflammatory mediators, interleukin (IL)-6 and IL-8, by oral epithelial cells stimulated by *F. nucleatum*. In a real-time reverse transcription-polymerase chain reaction and an enzyme-linked immunosorbent assay, live *F. nucleatum* induced high levels of gene expression and protein release of IL-6 and IL-8. The effects of MG and GA were examined by treating KB oral epithelial cells with MG and GA and stimulating them with *F. nucleatum*. MG and GA inhibited significantly the increases in the IL-6 and IL-8 gene and protein levels in a dose-dependent manner. These compounds also inhibited the growth of *F. nucleatum*. No visible effects of MG and GA on the adhesion and invasion of KB cells by *F. nucleatum* were observed. In conclusion, both MG and GA inhibit IL-6 and IL-8 production from *F. nucleatum*-activated KB cells.

***Keywords*:** methyl gallate, gallic acid, interleukin-6, interleukin-8, *F. nucleatum*

Periodontal diseases are multifactorial infections that are elicited by a complex of bacterial species that interact with host tissues and cells, inducing a range of pro-inflammatory products from various host cells. *Fusobacterium nucleatum* appears to interact with other periodontal bacteria by acting as a bridge between early and late colonizers, and mediating the shift from nonpathogenic to pathogenic microflora (Kolenbrander *et al*., 1995). *F. nucleatum* is a potentially important regulator of the host response due to its ability to invade cells and tissues and subsequently modify the immune response (Han *et al*., 2000). Epithelial cells act as a physical barrier and function as a sensor for the presence of bacteria. Direct physical contact between bacteria and the mucosal surface triggers the expression of a variety of immune response mediators from epithelial cells (Kagnoff and Eckmann, 1997; Okada and Murakami, 1998). One such modulator is interleukin (IL)-8, a low-molecular weight, pro-inflammatory chemokine that attracts and activates neutrophils. IL-8 expression has been suggested to be an important regulatory mechanism leading to neutrophil migration to the gingival sulcus (Bickel, 1993; Okada and Murakami, 1998). Several studies have reported that periodontal pathogens have various effects on the production of IL-8 from epithelial cells (Darveau *et al*., 1998; Huang *et al*., 1998). IL-6 plays important roles in the development of infectious diseases and is involved in both the local and systemic immune response to bacterial antigens (Van Snick, 1990). The regulation of inflammatory reactions in host cells has been suggested to be one way of controlling the progression of periodontitis (Takashiba *et al*., 2003). Recently, active compounds with the capacity to modulate the host inflammatory response have attracted considerable attention because they may represent potential new therapeutic agents for treating periodontal diseases (Paquette and Williams, 2000; Bodet *et al*., 2007).

Galla Rhois is an outgrowth of plant (*Rhus chinensis* L.) tissue caused by a mite parasite (*Schlechtendalia chinensis* Bell), and has been used as an antibacterial and anti-inflammatory drug in traditional Oriental medicine (Namba, 1993). Galla Rhois is rich in gallotannin, which is a mixture of polygalloyl esters of glucose. It was reported that extracts of Melaphis chinensis containing gallotannins, particularly methyl gallate (MG) and gallic acid (GA), had a significant inhibitory effect on the growth, water-soluble glucan synthesis and aggregation of *Streptococcus mutans* (Wu-Yuan *et al*., 1988). GA and its derivatives are natural polyphenyl products, and are particularly abundant in processed beverages, such as red wine and green tea (Graham, 1992). They have a wide range of biological activities, including anti-oxidant, anti-inflammatory, and anti-microbial activities (Kroes *et al*., 1992; Kubo *et al*., 2001). It was recently reported that MG and GA inhibited the growth of cariogenic and peiodonto-

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pathic bacteria as well as the *in vitro* formation of *S. mutans* biofilms (Kang *et al*., 2008). However, these studies did not report the effects of MG and GA on the inflammatory reaction by oral pathogenic bacteria. This study examined the effects of MG and GA on the production of inflammatory mediators, IL-6 and IL-8, by oral epithelial cells stimulated with *F. nucleatum.*

Materials and Methods

Materials
Methyl gallate (MG) and gallic acid (GA) were purchased from Sigma (USA).

Bacterial culture
Fusobacterium nucleatum ATCC 10953 was grown anaerobically in Trypticase soy broth supplemented with a yeast extract (1 mg/ml), hemin (5 μg/ml), and menadione (1 μg/ml) (Kang *et al*., 2005). The bacteria in the logarithmic growth phase were used in all experiments. For preparation of killed *F. nucleatum*, the bacteria were harvested, washed three times with phosphate-buffered saline (PBS), and resuspended in a small volume of PBS. The bacteria were then exposed to heat (100°C for 10 min), ultraviolet light (UV, a standard germicidal UV lamp for 3 h), or formalin (4% formalin for 20 min). The formalin-treated bacteria were washed extensively with PBS, and resuspended again in a small volume of PBS.

Cell culture and RNA extraction
KB cells, a human mouth epithelial cell line, were grown in 5% CO_2 at 37°C in minimal essential medium containing Earle's salts (Hyclone, USA), 2 mmol/L L-glutamine (MEM), 10% fetal bovine serum and 50 μg/ml gentamicin. The bacteria were washed twice with PBS and once with complete MEM. After resuspension in complete MEM, the optical density of the bacterial suspension was measured at 600 nm, and then diluted to an optical density of 0.5, which corresponded to 5×10^8 CFU/ml. 6-well plates were seeded with 3×10^5 KB cells and incubated with *F. nucleatum* [multiplicity of infection (MOI) of 1:100] for various times. The total RNA was isolated from the KB cells according to the manufacturer's specifications using an Easy-BLUE^{TM} RNA extraction kit (iNtRON Biotech, Korea).

Quantitative reverse transcription and polymerase chain reaction (RT-PCR)
The total RNA (1 μg) was heated to 70°C for 5 min and then chilled on ice. Each sample was reverse-transcribed to cDNA for 60 min at 42°C using a cDNA synthesis kit (Bioneer, Korea). Real-time RT-PCR was performed to quantify the mRNA expression of IL-6 and IL-8 using the Rotor-Gene 3000 system (Corbett Research, Australia) with a SYBR Green PCR kit (QIAGEN, Germany). cDNA samples were subjected to RT-PCR using the following primers for IL-8 (5'-TGTGCTCTCCAAATTTTTTTACTG-3'; 5'-CTCTCTTTCCTCTTTAATGTCCAGC-3'), IL-6 (5'-TCAATGAGGAGACTTGCCTG-3'; 5'-GATGAGTTGTCATGTCCTGC-3') and β-actin (5'-AGCGGGAAATCGTGCGTG-3'; 5'-CAGGGTACATGGTGGTGCC-3') (Na *et al*., 2001; Kang and Kuramitsu, 2002). The reactions were set at 95°C for 15 min to activate the polymerase, followed by 45 cycles of a 95°C for 10 sec, 59°C for 15 sec, and 72°C for 20 sec. The results of assays were normalized to cDNA mRNA. The relative ratio of target mRNA for each sample was calculated from its threshold cycle using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Cytotoxicity
The cytotoxicity of MG and GA were measured using a MTT assay, as described previously (Kang *et al*., 2008). The cells were treated with either MG or GA at concentrations of 0.2~1 mM and stimulated with *F. nucleatum* (MOI of 1:100) for 24 h. The cultures of the control group were left untreated. A MTT solution (5 mg/ml, Sigma) was added to each well of culture plate, which was then covered with aluminum foil for 4 h at 37°C under 5% CO_2 and 95% air. Consecutively, the medium was removed and DMSO (Sigma) was added to extract the MTT formazan. The absorbance of each well was measured at 540 nm using an automatic microplate reader.

Cytokine assay
The bacteria were washed twice with PBS and once with complete MEM. After resuspension in complete MEM, the optical density of the bacterial suspension was measured at 600 nm. Subsequently, the suspension was diluted to an optical density of 0.5, which corresponded to 5×10^8 CFU/ml. The KB cells were plated at 1×10^5 cells/well into 24-well plates. After 24 h, the cells were pretreated with various concentrations of MG or GA for 1 h, and then stimulated with *F. nucleatum* (MOI of 1:100). After 8 h incubation at 37°C, the culture supernatants were collected to determine the levels of IL-6 and IL-8 using an ELISA kit (BioLegend, USA) according to the manufacturer's directions.

Antibacterial assay
The Minimum Bactericidal Concentration (MBC) was the lowest concentration of the test compound that showed no visible growth in the drug-free cultivation. The bacterial cultures with MG or GA (0.04~5 mM) were withdrawn and dropped onto blood agar plates. The assays were performed at least in triplicate on separate occasions.

Adhesion test
The adhesion test was carried out as described previously (Kang *et al*., 2005). The adhesion test was performed in a 4 well Lab-Tek II chamber slide system (Nalge Nunc International, USA). Briefly, the KB cells were grown until they reached confluence (1×10^5 cells/well) for assays. The cells were then pre-treated with MG and GA for 1 h, and bacteria were added at a MOI of 100. In order to allow attachment, the plates were incubated in 5% CO_2 at 37°C for 1 h. The cells were washed three times with PBS, fixed with methanol, Giemsa (Sigma) stained for 30 min, washed with PBS, and air dried. The adhesion of bacteria on epithelial cells was observed using a bright-field microscope.

Invasion assay
Invasion assays were carried out using a conventional anti-

biotic protection assay (Deshpande *et al*., 1998) with some modifications. Briefly, the KB epithelial cells were grown to near confluence (1×10^5 cells/well) for the assays. The cells were pretreated with either MG or GA (1 mM) for 1 h. The bacteria were then added at a MOI of 100 for 4 h and incubated at 37°C in an atmosphere containing 5% CO_2. The monolayers were washed twice with PBS, and fresh medium containing gentamicin (300 μg/ml) and metronidazole (200 μg/ml) were then added. The monolayers were incubated for an additional 1 h to kill the extracellular bacteria. After washing twice with PBS, the cells were lysed with 1 ml of distilled water for 30 min. For bacterial counting, real-time PCR was carried out using a Rotor-Gene 3000 system employing a QuantiTect Probe PCR Master Mix (QIAGEN). Twenty five microliter of a mixture containing 3 μl of DNA template, 12.5 μl of 2× QuantiTect Probe PCR Master Mix, sense and antisense primers (10 pmol each), dual-labeled probe (10 pmol) and sterile distilled water were placed into a tube. The *F. nucleatum*-specific primers and dual-labeled probe sets used for real-time PCR were as follows: primers 5'-CGCAGAAGGTGAAAGT CCTGTAT-3' and 5'-TGGTCCTCACTGATTCACACAGA-3'; and probes FAM-ACTTTGCTCCCAAGTAACATGGAACA CGAG-BHQ. The primers and dual-labeled probes were synthesized commercially with some modifications (Operon Biotechnologies, Germany) (Suzuki *et al*., 2004). The reaction conditions were set to 95°C for 15 min, followed by 50 cycles of 95°C for 15 sec and 58°C for 1 min. *F. nucleatum* in the tested samples was quantified using the standard curves generated from standard bacterial DNA dilutions. In addition, each reaction was performed using standard bacterial DNA dilutions for absolute quantification. Each PCR was carried out in duplicate. The gene expression levels were calculated using complementary computer software (Corbett Research). The data are expressed as the percentage of the initial inoculums recovered after washing or antibiotic treatment.

Statistics

Each experiment was carried out in triplicate, and the mean value was analyzed further. Statistical analysis was carried out using SPSS version 17.0 (Statistical packages for Social Science version 17.0; SPSS Inc., USA). A Mann-Whitney test was used to identify any statistically significant differences in the experiments.

Results

mRNA and protein synthesis of IL-6 and IL-8 by KB cells stimulated with *F. nucleatum*

KB oral epithelial cells were stimulated with *F. nucleatum* (MOI=1:100) for 2, 4, 8, and 24 h, after which IL-6 and IL-8 mRNA expressed by the cells were quantified by real-time RT-PCR (Fig. 1A). The IL-8 and IL-6 mRNA in KB cells were approximately 2-fold to 3-fold induced by the live *F. nucleatum* in the early stages (2 h) after infection ($P<0.05$). The levels of both IL-6 and IL-8 mRNA induced

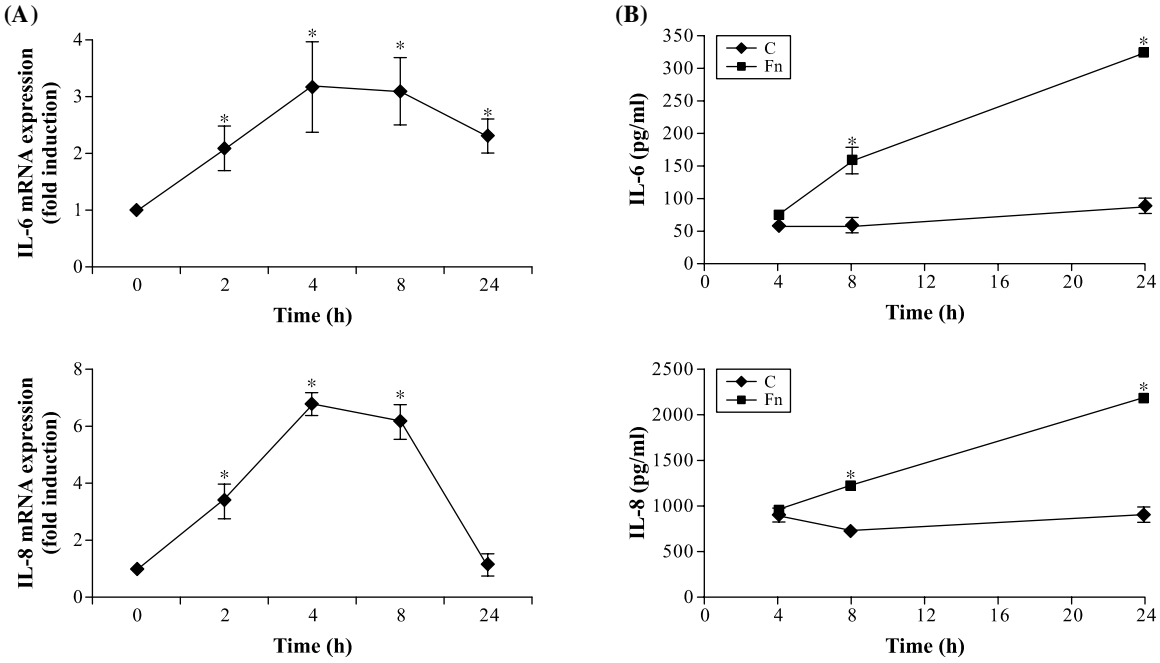

Fig. 1. mRNA and protein synthesis of IL-6 and IL-8 by KB cells stimulated with *F. nucleatum*. (A) The isolated mRNA of each time point was reverse transcribed and the cDNA samples were amplified using real-time quantitative PCR and SYBR Green detection as described in 'Materials and Methods'. The amount of mRNA expressed by control cells was designated as 1.0, and relative expression amounts are shown as fold induction. (B) Time course of the *F. nucleatum*-induced production of IL-6 and IL-8 in KB cells. IL-6 and IL-8 concentrations of the culture supernatants were measured by ELISA. The data are reported as Mean±SD of a representative experiment performed in triplicate. C, control cells without stimulation of *F. nucleatum*; Fn, cells stimulated with *F. nucleatum* *$P<0.05$, *F. nucleatum* vs. control.

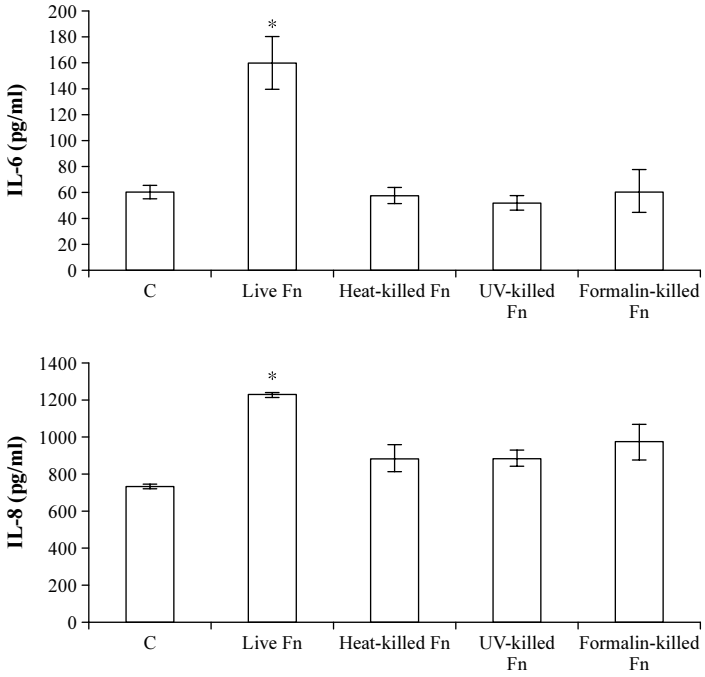

Fig. 2. Comparison of the live and killed *F. nucleatum* for IL-6 and IL-8 production by KB cells. 1×10^5 KB cells were seeded in 24-well plates. After 24 h, the cells were incubated with live, heat-killed, UV-killed, or formalin-killed *F. nucleatum* at a MOI of 1:100 in a final volume of 0.5 ml for 8 h. IL-6 and IL-8 concentrations of culture supernatants were measured by ELISA. The data are expressed as Mean±SD of a representative experiment performed in triplicate. C, control cells without stimulation of *F. nucleatum*; Fn, cells stimulated with *F. nucleatum*. $^*P<0.05$, live *F. nucleatum* vs. control.

by *F. nucleatum* peaked at 4 h, followed by a slow decrease. To evaluate the time course of IL-6 and IL-8 release, the KB cells were stimulated with *F. nucleatum* for 4, 8, and 24 h, after which the level of IL-6 and IL-8 released was determined by ELISA. The expression of IL-6 and IL-8 increased significantly over time with microbial stimulation ($P<0.05$). The level of IL-6 protein release was low at 4 h and increased significantly after 8 h. The release of IL-8 followed a similar pattern to that observed with the release of IL-6 in KB cells stimulated with *F. nucleatum* (Fig. 1B).

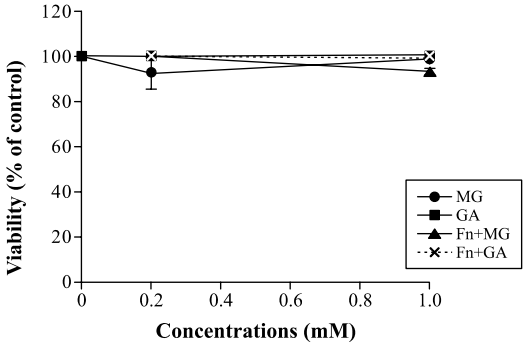

Fig. 3. Cytotoxicity of MG or GA on KB cells. The KB cells were treated with MG or GA (0.2~1 mM) and stimulated with *F. nucleatum* (MOI=1:100) for 24 h. Untreated cells were used as a control. The cell viability was assessed using an MTT assay. The data are expressed as Mean±SD of a representative experiment performed in triplicate. Fn, *F. nucleatum*; MG, KB cells treated with methyl gallate; GA, KB cells treated with gallic acid; Fn+MG, KB cells treated with methyl gallate and stimulated with *F. nucleatum*; Fn+GA, KB cells treated with gallic acid and stimulated with *F. nucleatum.*

Comparison between live and killed bacteria

To determine if viable bacteria were required for the IL-6 and IL-8 induction, the KB cells were incubated with same number of live or killed *F. nucleatum* (MOI=100) for 8 h. The IL-6 level induced by heat-killed, UV-killed and formalin-killed bacteria were at most half of those achieved by live bacteria ($P<0.05$) (Fig. 2). The IL-8 level induced by live bacteria also was higher than that induced under the other conditions ($P<0.05$). Therefore, bacterial viability is essential in *F. nucleatum*-induced IL-6 and IL-8 production in KB cells.

Cytotoxicity of MG and GA

The viability of KB cells incubated with MG at concentrations of 0.2 mM and 1 mM for 24 h were 92.5±7.1% and 99.2±1.2% of the control value, respectively. GA (0.2~1 mM) showed no obvious cytotoxic effects. In addition, the viability of KB cells with MG or GA stimulated with *F. nucleatum* were not changed (Fig. 3).

Effect of MG and GA on mRNA and protein synthesis of IL-6 and IL-8

KB cells were stimulated with *F. nucleatum* in the presence

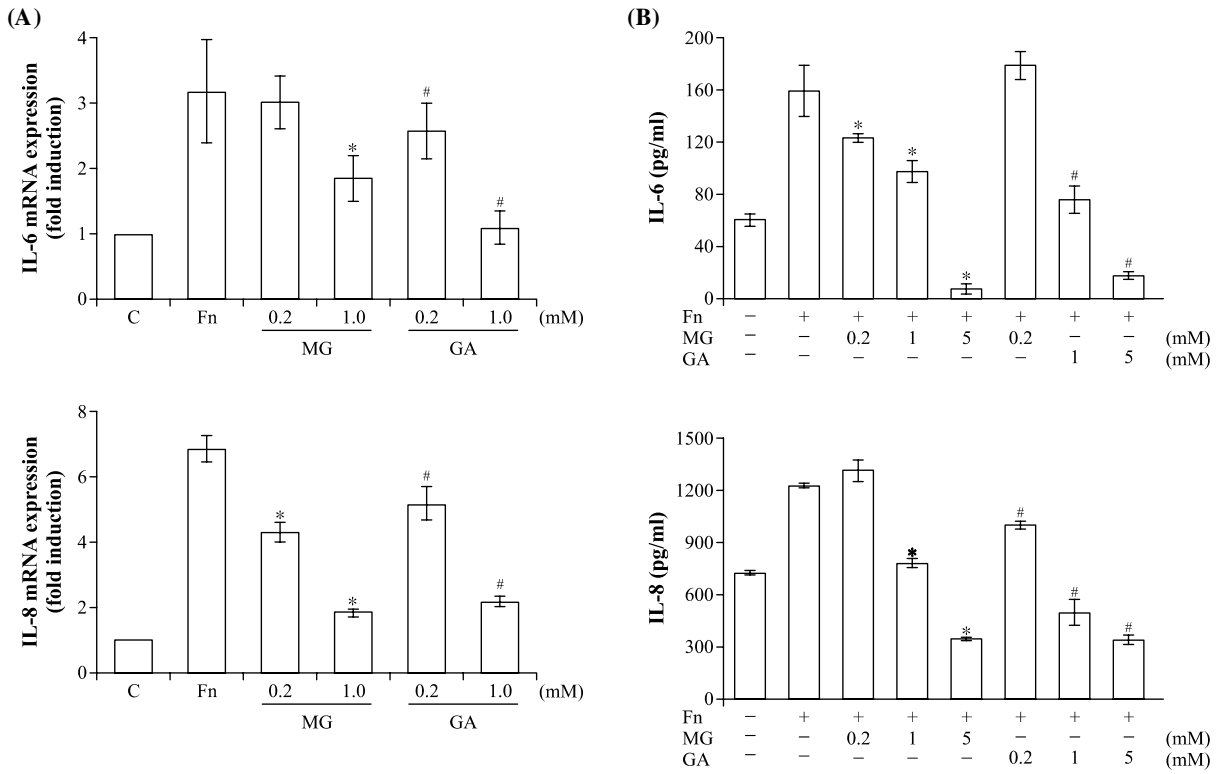

Fig. 4. Effect of MG and GA on IL-6 and IL-8 mRNA and protein synthesis in KB cells stimulated with *F. nucleatum*. (A) KB cells were treated with the indicated concentration of MG and GA for 1 h before being stimulated with *F. nucleatum* (1:100) for 4 h. The total RNA was isolated, and the mRNA expressions for IL-6 and IL-8 were quantified using real-time RT-PCR. The amount of mRNA expressed by control cells was designated as 1.0, and relative expression amounts are shown as fold induction. (B) KB cells were treated with the indicated concentrations of MG and GA for 1 h before being infected with *F. nucleatum* (1:100) for 8 h. The secretion of IL-6 and IL-8 was measured by ELISA, as described in 'Materials and Methods'. The data are expressed as Mean±SD of a representative experiment performed in triplicate. C, control cells without stimulation of *F. nucleatum* in the absence of MG or GA; Fn, cells stimulated with *F. nucleatum* in the absence of MG or GA. **P*<0.05, cells stimulated with *F. nucleatum* in the presence of MG vs. *F. nucleatum* control; #*P*< 0.05, cells stimulated with *F. nucleatum* in the presence of GA vs. *F. nucleatum* control.

or absence of MG or GA for 4 h, after which IL-6 and IL-8 mRNA expressed by the cells were quantified by real-time RT-PCR. Both MG and GA (0.2∼1 mM) suppressed the *F. nucleatum*-induced expression of the IL-6 and IL-8 genes in a dose-dependent manner ($P<0.05$) (Fig. 4A). IL-6 and IL-8 secretion were measured by ELISA to confirm the correlation of mRNA expression with protein production (Fig. 4B). Stimulation of KB cells with *F. nucleatum* during 8 h induced the secretion of both cytokines. Treatment with MG and GA blocked the secretion of IL-6 and IL-8 induced by *F. nucleatum* in a dose-dependent manner (0.2 mM MG, 22.7±7.7%; 1 mM MG, 38.7±2.6%; 5 mM MG, 96.3±2.0%; 1 mM GA, 52.6±0.6%; 5 mM GA, 89.4± 0.5% inhibition in IL-6; 1 mM MG, 36.2±1.6%; 5 mM MG, 72.0±0.7%; 0.2 mM GA, 18.6±1.0%; 1 mM GA, 59.9±5.8%; 5 mM GA, 72.7±1.8% inhibition in IL-8) ($P<0.05$).

Determination of the antibacterial activity of MG and GA against *F. nucleatum*

Fig. 5 shows the bactericidal effects of MG or GA (0.02∼5 mM) against *F. nucleatum*. The MBC against *F. nucleatum* were 1 mM (MG) and 5 mM (GA), respectively. MG exhibited more potent inhibitory activity against the growth of *F. nucleatum* than GA.

Effect of MG and GA on cell attachment/invasion by *F. nucleatum*

Cell attachments were performed to determine if MG or GA affects the cell attachment of *F. nucleatum* to KB cells. Both MG and GA had little or no effect on the cell attachment of *F. nucleatum* to KB cells (Fig. 6A). In addition, MG and GA had no effect on the invasion of *F. nucleatum* to KB cells (Fig. 6B).

Discussion

Periodontal diseases result from the complex actions of a group of periodontal bacteria, mostly Gram-negative anaerobes. *F. nucleatum* is the most numerous Gram-negative bacterium in the healthy oral cavity, but the mass of *F. nucleatum* increases significantly during active periodontal disease (van Winkelhoff *et al*., 2002). Interactions between the periodontal bacteria and human oral epithelial cells lead to the activation and expression of proinflammatory cytokines including IL-6 and IL-8 in epithelial cells (Kornman *et al.*, 1997). IL-6, a multifunctional cytokine, plays an important

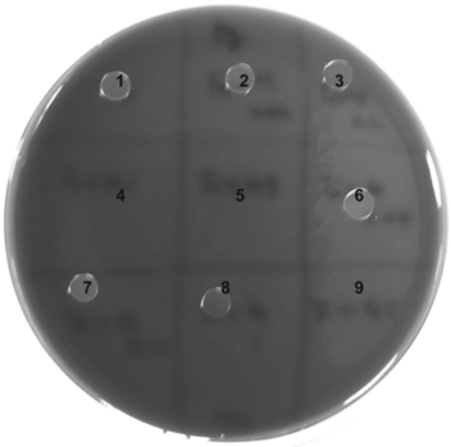

Fig. 5. Bactericidal effects of MG and GA against *F. nucleatum.* Serial doses of MG and GA (0.04, 0.2, 1 or 5 mM) were added to the bacterial cultures in 96-well plates and incubated. After 24 h, each bacterial culture (5 μl) was dropped onto blood agar plates and incubated anaerobically. Visible growth indicates white-colored colony. No growth was shown in the *F. nucleatum* culture with MG (1∼5 mM) and GA (5 mM). 1, untreated *F. nucleatem* culture; 2, *F. nucleatum* culture with 0.04 mM MG; 3, *F. nucleatum* culture with 0.2 mM MG; 4, *F. nucleatum* culture with 1 mM MG; 5, *F. nucleatum* culture with 5 mM MG; 6, *F. nucleatum* culture with 0.04 mM GA; 7, *F. nucleatum* culture with 0.2 mM GA; 8, *F. nucleatum* culture with 1 mM GA; 9, *F. nucleatum* culture with 5 mM GA.

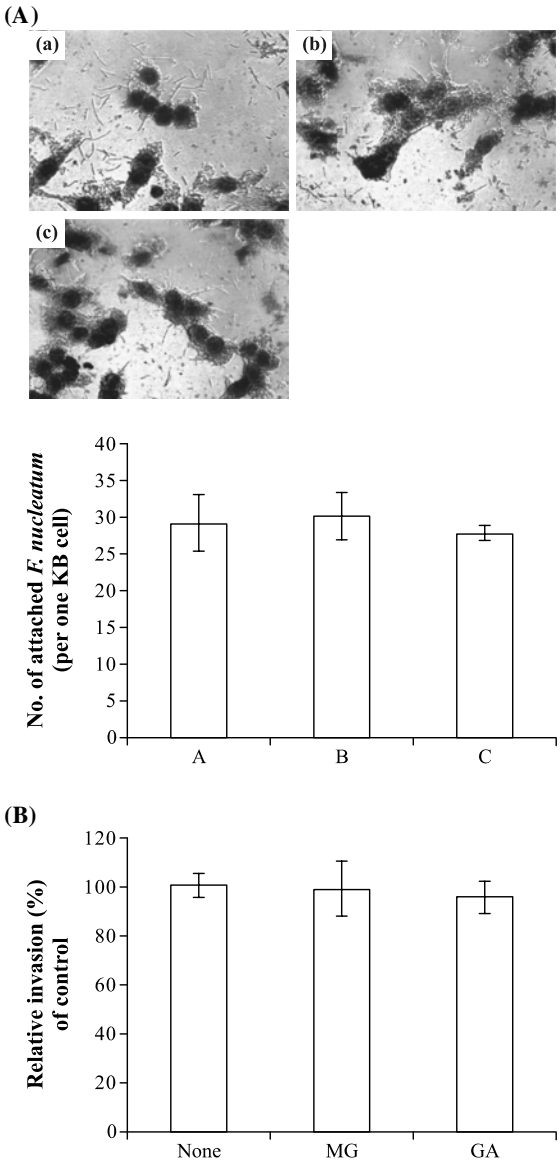

Fig. 6. Effect of MG and GA treatment on the attachment (A) and invasion (B) of *F. nucleatum* to KB human mouth epithelial cells. KB cells were pre-treated with MG (1 mM) or GA (1 mM) and stimulated with *F. nucleatum,* as described in 'Materials and Methods'. (a) untreated *F. nucleatum* vs. KB cells; (b) MG treated *F. nucleatum* vs. KB cells; (c) GA treated *F. nucleatum* vs. KB cells. Magnification, ×1,000. The level of invasion is expressed as a percentage of the control level without any materials. The data are expressed as Mean±SD of a representative experiment performed in triplicate.

role in regulating the immune response during periodontal disease (Van Snick, 1990). IL-6 expression was found to be higher at sites of periodontal inflammation and closely related to the clinical severity of periodontitis (Irwin and Myrillas, 1998). IL-8, a C-X-C chemokine, is up-regulated in oral or gingival epithelial cells after a challenge with several periodontal bacteria, such as *Actinobacillus actinomycetemcomitans*, *F. nucleatum*, *Eikenella corrodens*, and *Prevotella intermedia* (Yumoto *et al*., 1999; Han *et al.,* 2000; Sfakianakis *et al*., 2001). Several reports also suggest that cytokines play important roles in the pathogenesis of periodontitis (Kjeldsen *et al*., 1993; Wilson *et al*., 1996). IL-8 has been shown to be localized in gingival tissue sections of patients with periodontitis, and the levels of IL-8 mRNA were shown to correspond to the severity of periodontitis (Tonetti *et al*., 1994). IL-8 is a potent chemokine that directs the migration of polymorphonuclear leukocytes, monocytes, and macrophages to the site of an infection. Increased levels of IL-8 are found in the gingival crevicular fluid of inflamed periodontal sites compared with healthy sites. Periodontal therapy reduces immune cell numbers in the infiltrate and the levels of IL-8, suggesting a relationship between this chemokine and periodontal status. In this study, MG and GA exhibited the capacity to inhibit *F. nucleatum*-induced IL-8 production by epithelial cells. This suggests that MG and GA may contribute to reduce the afflux of inflammatory cells at the site of infection.

This study showed that IL-8 mRNA in KB cells was up-regulated by *F. nucleatum* in the early stage (2 h) after an infection. In addition, *F. nucleatum* strongly stimulated the production of IL-6 and IL-8. These results are consistent with clinical studies demonstrating that *F. nucleatum* is quite prevalent during the early stages of inflammation associated with gingivitis (Moore and Moore, 1994). This study also examined whether viable *F. nucleatum* is essential for the induction of cytokine expression in KB cells. Significantly higher levels in IL-6 and IL-8 were detected after exposing the KB cells to live *F. nucleatum* than to killed *F. nuclea-*

tum-infected cells. These observations corresponded to the findings that *F. nucleatum* is highly invasive for KB cells and a potent stimulator of IL-8 expression (Han *et al*., 2000). This may be related to the fact that *F. nucleatum* has fimbriae. The presence of fimbriae probably makes *F. nucleatum* adept at adhesion to and internalization by epithelial cells, during which strong IL-6 and IL-8-inducing stimuli may be generated.

Some studies reported the antimicrobial effects of Galla Rhois-derived tannins (MG and GA) (Ahn *et al*., 1998; Kang *et al*., 2008). In addition, many authors reported that gallotannin and other tannins have a variety of biological effects ranging from anti-inflammatory to anticancer and antiviral effects (Mota *et al*., 1985; Uchiumi *et al*., 1996; Feldman *et al*., 2001). The mechanisms underlying the anti-inflammatory effect of tannins include the scavenging of radicals (antioxidant effect) (Hagerman *et al*., 1999) and inhibiting the expression of inflammatory mediators, such as some cytokines (Feldman *et al*., 2001). In this study, KB oral epithelial cells were stimulated with *F. nucleatum* after a MG or GA treatment to determine if MG and GA can reduce the production of pro-inflammatory mediators. Both MG and GA inhibited IL-6 and IL-8 gene expression by *F. nucleatum* in a dose-dependent manner. In addition, MG and GA showed the capacity to inhibit *F. nucleatum*-induced IL-6 and IL-8 secretion by oral epithelial cells.

The adhesion and invasion of epithelial cells are essential steps in pathogenesis. The adhesion of microbes to host mucosal surfaces is a major determinant of successful colonization and infection. Therefore, the effects of MG or GA on the adhesion and invasion of *F. nucleatum* to KB epithelial cells were examined. However, compared with the controls, there was no significant decrease in epithelial cell adhesion and invasion by *F. nucleatum* after MG or GA exposure. The therapeutic strategies for the treatment of periodontal disease have been directed towards two different and complementary paths: antimicrobial therapy and host modulation. When faced with a bacterial infection, antibiotic therapy is normally used to reach the MBC or Minimum Inhibitory Concentration (MIC) in an attempt to eliminate the virulence of microorganisms. In this study, both MG and GA exhibited dual actions as inhibitors of the mediators of inflammation as well as showing antibacterial activities. MG and GA inhibited the *F. nucleatum*-induced release of IL-6 and IL-8 from human mouth epithelial cells. The MBC against *F. nucleatum* was 1 and 5 mM for MG and GA, respectively. These compounds have distinct advantages. They not only kill microorganisms but also inhibit the inflammatory mediators induced by the pathogen. Therefore, they affect both the source and vehicle of inflammation. This would decrease the overall bacterial load and reduce the up-regulation of pro-inflammatory mediators mediated by bacteria.

Most cellular studies examined the mechanism for the anti-inflammatory effects. Erdèlyi *et al*. (2005) suggested that the inhibitory effect of gallotannin on inflammatory cytokines, including IL-8, was related to the blocking of activator protein 1 and nuclear factor-κB (NF-κB) activation. Kim *et al*. (2006) also suggested that GA inhibits IL-6 production in mast cells by attenuating the activity of NF-κB and inhibiting the activity of the p38 mitogen-activated protein kinase pathways. However, the present study focused only the both antimicrobial and anti-inflammatory activities of MG and GA *in vitro*.

In conclusion, MG and GA can inhibit the production of IL-6 and IL-8 from human mouth epithelial cells induced by *F. nucleatum* as well as the growth of *F. nucleatum*. This suggests that both MG and GA are good candidates for controlling periodontal disease. Further studies on the action mechanisms of these compounds will be needed to determine their future therapeutic use. In addition, *in vivo* studies will be necessary to evaluate the efficacy and safety for oral use.

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