치의과학 박사 학위논문

In vivo Inhibition of *Porphyromonas gingivalis* Growth and Prevention of Periodontitis With Quorum-Sensing Inhibitors

쿼럼 센싱 억제제를 이용한 Porphyromonas gingivalis 성장 억제 및 치주질환 예방에 대한 *in vivo* 연구

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Abstract

In vivo Inhibition of *Porphyromonas gingivalis* Growth and Prevention of Periodontitis With Quorum-Sensing Inhibitors

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Background: Autoinducer (AI)-2 has an important role in biofilm formation in the oral environment. Mature biofilms formed as a result of the cell to cell communication make it difficult to overcome periodontitis with the use of antibiotics. Previous in vitro studies suggest that quorum-sensing inhibitors (QSIs) interfere with AI-2. This study compared the QSI effects resulting from an oral inoculation of *Porphyromonas gingivalis* in an experimental animal model.

Methods: Forty-five male mice were divided into three groups (n = 15 each): 1) infection, 2) QSI, and 3) control. Infection and QSI groups received oral inoculation of *P. gingivalis*, whereas treatment with QSIs (furanone compound and D-ribose) was only performed in the QSIs group. The control group was a negative control not receiving manipulation. After 42 days, mice were sacrificed, and the distance from the alveolar bone crest (ABC) to the cemento-enamel junction (CEJ) was measured by microcomputed tomography. *P. gingivalis* DNA was quantified in the soft and hard tissues around the molar teeth by real-time polymerase chain reaction.

Results: Distance from ABC to CEJ was significantly increased in the *P. gingivalis* infection group compared with the control group (P = 0.02) and significantly decreased in the QSI group compared with the infection group (P = 0.02). The QSI group contained 31.64% of the bacterial DNA count of the infection group.

Conclusion: Use of QSIs in the mice infection model showed a reduction of bone breakdown and a decrease in the number of bacteria *in vivo*, suggesting that QSIs can be a new approach to prevention and treatment of periodontitis.

KEY WORDS

Biofilms; models, animal; *Porphyromonas gingivalis*;

Quorum sensing; ribose.

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CONTENTS

I. INTRODUCTION

Bacteria perform particular behaviors only when living in a community and not in isolation (Taga and Bassler 2003). Bacterial phenotypes depend on gene expression, which can be stimulated by cell to cell signaling between other microorganisms (Raj and van Oudenaarden 2008). Through cell to cell communication, bacteria regulate the following: 1) bioluminescence, 2) secretion of virulence factors, 3) biofilm formation, 4) sporulation, 5) conjugation, and 6) pigment production, all of which can be harmful to humans (Fuqua, Winans et al. 1996, Bassler 1999, Miller and Bassler 2001). This is conducted by the accumulation and interaction with small extracellular molecules known as autoinducers (AIs). AI-2 quorum sensing (QS) is a major QS signal molecule in which the bacteria either maintains the population density or triggers active proliferation to achieve a quorum (Taga and Bassler 2003, Hardie and Heurlier 2008). In particular, AI-2 causes cell to cell communication between oral bacteria and plays an important role in biofilm formation (Kolenbrander, Palmer et al. 2006, Shao and Demuth 2010).

Infections associated with biofilm have been estimated to mediate >65% of all chronic infections in humans (Ymele-Leki and Ross 2007, Cos, Tote et al. 2010). Periodontitis and dental caries are representative oral diseases that are generally acknowledged to be associated with oral

biofilm (Darveau, Tanner et al. 1997, Cvitkovitch, Li et al. 2003). Oral biofilm shows resistance to antibiotics. Estimates of 1,000 to 1,500 times greater resistance for biofilm-grown cells compared with planktonic grown cells have been suggested (Costerton, Stewart et al. 1999), and a subgingival mature biofilm needs 250 times greater concentration of antibiotics than the planktonic state for treatment (Sedlacek and Walker 2007). Because of this resistance, it is not easy for antibiotics to penetrate the thick structure of mature biofilms and achieve a therapeutic effect (Hoiby, Bjarnsholt et al. 2010).

For maturation of oral biofilm, AI-2 of *Fusobacterium nucleatum* plays a bridging role that links early colonizing commensals and late pathogenic colonizers. A previous in vitro study revealed that *F. nucleatum* AI-2 contributes to the interspecies interaction between the so-called "red complex" (*Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia*) (Socransky, Haffajee et al. 1998). Conversely, AI-2 of *F. nucleatum* could play an inhibitory effect on *Streptococcus oralis* (Jang, Sim et al. 2013). The action of QS and biofilm formation has been shown to be weakened by QS inhibitors (QSIs) (Lonn-Stensrud, Petersen et al. 2007, Jiang and Li 2013). Jang *et al.* suggested in an in vitro study those two QSIs, furanone compound [(5Z)-4-bromo-5-(bromomethylene)-2(5H)-furanone] and D-ribose, were shown to significantly inhibit biofilm growth of three different species of bacteria: 1) *F. nucleatum*, 2) *P. gingivalis*, and 3) *T. forsythia* (Jang, Choi et al. 2013). No undesirable cytotoxic effects or induction of pro-inflammatory factors were

observed. QSIs disrupt the signaling and colonization between bacteria and prevent increases in cell density (Jang, Choi et al. 2013). By disrupting the biofilm, thereby making the bacteria more susceptible to traditional antibiotics, these QSIs may provide the new therapeutic approach against infections or diseases involving drug-resistant bacteria (Martin 2009). Among the QSIs, furanone compound and D-ribose were the most remarkable AI-2 inhibitors that have been discovered to date (Manefield, Rasmussen et al. 2002, Baveja, Li et al. 2004, Jang, Choi et al. 2013). It was hypothesized that QSIs would inhibit the action of *P. gingivalis* AI-2 and reduces the biofilm formation and co-aggregation and the progress of periodontitis in a mice model. To prove this hypothesis, experimental periodontitis with *P. gingivalis* inoculation was induced, and the efficacy of two QSIs, furanone compound and D-ribose, were tested on biofilm formation and the expression of periodontitis.

II. MATERIALS AND METHODS

Animals

All procedures were conducted following the guidelines of National Institutes of Health for the Care and Use of Laboratory Animals. The protocol for animal maintenance and all experimental procedures were approved by the Institutional Animal Care and Use Committee of Seoul National University, Seoul, Korea (SNU-140618-1). Forty-five male BALB/c mice (8 weeks old; 200g) were used. Animals were kept in a specific pathogen-free room in which the indoor temperature was maintained at 23 to 25° C with a 12-hour light/dark cycle.

Bacterial Strain and Growth Conditions

P. gingivalis (ATCC 33277) was grown in a brain-heart infusion medium that was supplemented with 10 mg/mL hemin (Sigma-Aldrich, St. Louis, MO) and 0.2 mg/mL vitamin K (Sigma-Aldrich, St. Louis, MO). Bacteria were grown in an anaerobic chamber (5% H_2, 10% CO_2, and 85% N_2) at 37°C for 18 hours.

Quorum Sensing Inhibitors

A furanone compound (Sigma-Aldrich, St. Louis, MO) and D-ribose

(Tokyo Chemical Industry, Tokyo, Japan) were used as AI-2 QSIs. The furanone compound was synthesized based on the method by Manny *et al* (Manny, Kjelleberg et al. 1997).

Experimental Design

A modified mouse model was used to reproduce experimental periodontitis (Baker, Evans et al. 1994). Animals were given sulfamethoxazole (1 mg/mL) (Sigma-Aldrich, St. Louis, MO) and trimethoprim (Sevatrim, Swiss Pharmaceutical, Tainen City, Taiwan) (200 mg/mL) in their drinking water ad libitum for 10 days, thus inhibiting commensal bacteria. Antibiotics were administered by mixing with 463 mL deionized water. Because of light sensitivity, the water bottle was covered with aluminum foil to protect from the light. Animals were given a 4-day resting period without antibiotics after 10 days of antibiotics application.

Mice were randomized into three groups (n = 15 each): 1) infection, 2) QSIs, and 3) control. For the infection group, a 100-mL mixture of 2% carboxymethyl cellulose sodium (Tokyo Chemical Industry, Tokyo, Japan) (CMC) and *P. gingivalis* (5.0 X 10^9 cells/mL) was administered orally and applied topically to the anus at each treatment. CMC was expected to be sustained longer in the oral cavity (O'Brien-Simpson, Pathirana et al. 2005, Kesavalu, Sathishkumar et al. 2007, Verma, Rajapakse et al. 2010). For the QSI group, a 100 mL mixture of 2% CMC, *P. gingivalis* (5.0 X 10^9

cells/mL), and 20 mM furanone compound was orally administered and topically applied anally. D-Ribose was mixed with drinking water (50 mM D-ribose in 500 mL drinking water) for daily administration. Although D-ribose itself is water soluble, furanone needs to be mixed with alcohol, and if furanone mixed with alcohol was added to drinking water, the side effects of alcohol abuse may have surfaced (Liberman, Pilau et al. 2011, Porto, Semenoff Segundo et al. 2012). Therefore, D-ribose was mixed with drinking water, whereas furanone with alcohol was orally inoculated by injection form with CMC. For the control group, a 100 mL mixture of 2% CMC and phosphate-buffered saline was orally administered and topically applied anally each treatment.

Each group received administration of oral solution injection 10 times at 48 hour intervals by a 1 mL syringe. One additional administration was applied at 5 days before the animals were sacrificed (Figure 1). Rodents are coprophagic in nature, and this trait can create a cycle of oral reinfection. They intake their feces, and bacteria can return to their oral cavity (Rajapakse, O'Brien-Simpson et al. 2002, Lalla, Lamster et al. 2003). Forty-two days after the first gavage, all mice were anesthetized by intraperitoneal injection (Zoletil 50, Virbac Laboratories, Carros, France) (2% Rompun, Bayer Korea, Ansan, Korea) before the animals were sacrificed. Mice were sacrificed by placing them in a CO_2 chamber to harvest maxillary and mandibular tissues (Baker, Evans et al. 1994).

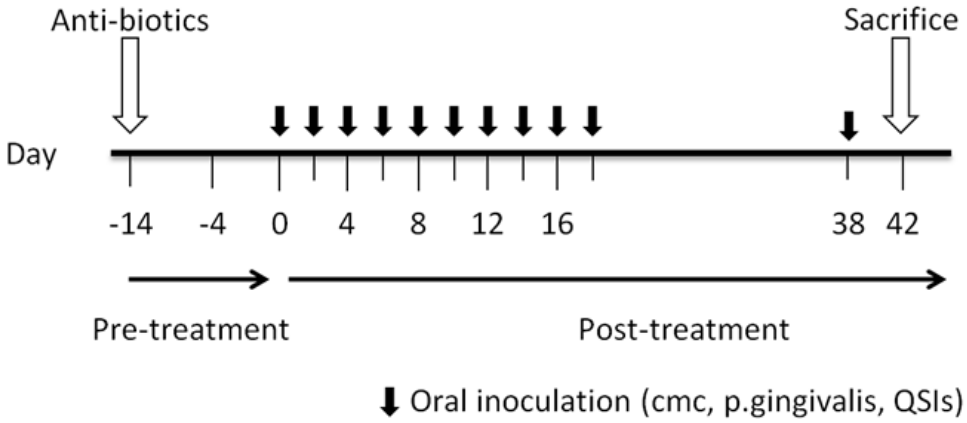

Figure 1. Study design

Animals were given an antibiotic for 10 days and 4-day resting period before the bacteria inoculation begins. Every 48 hours, the animals received oral administration of bacteria and QSIs with 10 times, and one additional administration was applied at 54 days

Maxillary tissue and teeth were separated using a surgical saw for micro computed tomography (micro-CT) analysis; mandibular tissues and teeth were also separated for polymerase chain reaction (PCR) analysis. For this, mandibular tissue and teeth were grinded together because biofilm could attach to the surface of the tooth.

Three-Dimensional (3D) Micro-CT Analyses of the Interproximal Area

A micro-CT scanner (SkyScan-1173, Bruker-MicroCT, Kontich, Belgium) was used to examine tissues that were harvested from the maxillary molar. Tissues were fixed in place using a positioner and scanned every 0.2° for >240° in the direction of the major axis of the sample. Each specimen was scanned 1,200 times. All two-dimensional (2D) images were then scanned and saved as TIF files (1,120 X 1,120 pixels). For the micro-CT data that were converted to 2D images, maxillary first molar (M1) and maxillary second molar (M2) were set in parallel (Dataviewer program, Bruker-MicroCT), and the resulting binary images were saved.

The distance from the alveolar bone crest (ABC) to the cemento-enamel junction (CEJ) of the interproximal site between M1 and M2 was measured (CT Analyzer v.1.13.5.1, Bruker-MicroCT).
For this measurement, the buccal surface of M1 and M2 was used as the reference point. The shortest distance from the ABC to the line connecting the adjacent CEJs between M1 and M2 was measured (Figure 2). Among the sagittal images, the image that showed the most recession of alveolar bone was selected for measurement.

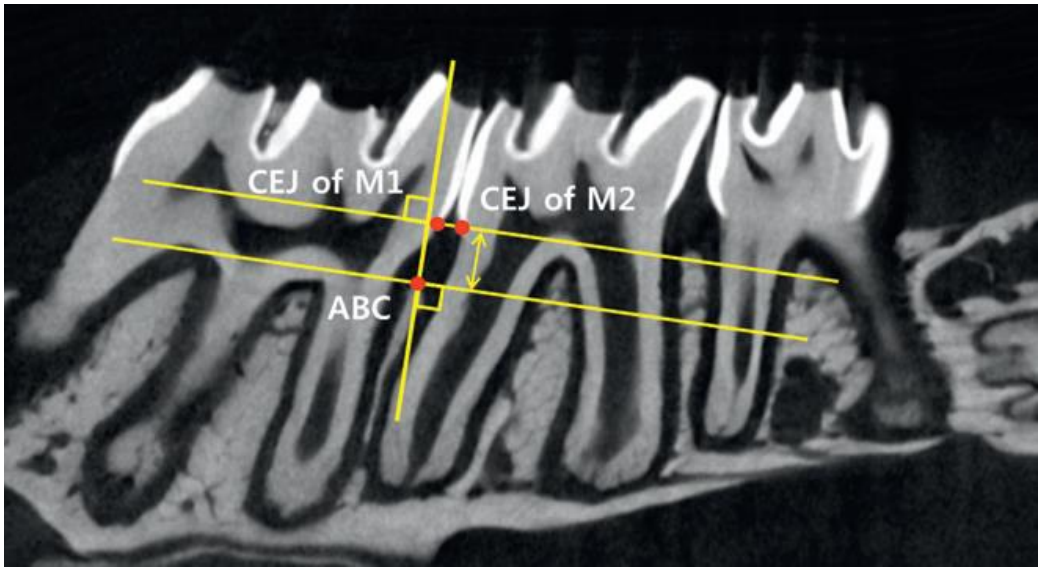

Figure 2. Measurement method of 3D micro-CT images.

A line is drawn to connect the CEJs of adjacent M1 and M2, and the shortest distance between this line and the ABC is measured.

Real-Time PCR

Soft tissue, tooth, and mandibular bone were taken from the left side of each mouse. To acquire biofilm grown cells, a tooth and soft tissue around the tooth were harvested using a surgical blade. The tissue samples were finely ground and then mixed. Next genomic DNA (gDNA) was extracted from 0.15 g tissue using a DNA extraction kit (Bacteria Genomic DNA Extraction Kit, iNtRON Biotechnology, Seoul, Korea) and quantified with a spectrophotometer (ND-2000 NanoDrop spectro-photometer, Thermo Fisher Scientific, Waltham, MA). Next, real-time PCR was performed on a real-time PCR system (7500 Real-Time PCR System, Thermo Fisher Scientific, Grand Island, NY) using 100 ng gDNA. DNA levels were normalized to those of mouse GAPDH.

The following mouse GAPDH primer sequences were used: 1) forward, 59-AGG TCG GTG TGA ACG GAT TTG-39, and 2) reverse, 59-TGT AGA CCA TGT AGT TGA GGT CA-39. The following *P. gingivalis* primer sequences were used: 1) forward, 59-TGC AAC TTG CCT TAC AGA GG-39, and 2) reverse, 59-ACT CGT ATC GCC CGT TAT TC-39. Transcript was determined using a PCR kit (QuantiFast SYBR Green PCR Kit, Qiagen, Hilden, Germany) with a program consisting of warming to 50°C for 2 minutes. Initial heat activation was at 95°C for 5 minutes. The two-step cycling was followed by: 1) 40 cycles of denaturation at 95°C for 10 seconds, 2) combined annealing at 60°C for 30 seconds, and 3) extension at 60°C for 30 seconds. A final extension was at 72°C for 10

minutes. Real-time PCR data were analyzed with the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). This method enabled the relative levels of DNA to be compared between the QSI and infection group.

Statistical Analyses

All statistical analyses were performed (R v.2.15.3, R Foundation for Statistical Computing, Vienna, Austria), and a P value < 0.05 was considered statistically significant. To estimate the mean distance from the CEJ to the ABC as assessed by micro-CT in the three experimental groups, data were analyzed with a linear effects model with the treatment methods set as fixed factors. The linear-effects model was adjusted using a generalized least-square estimation method that accounts for heteroscedasticity.

III. RESULTS

P.gingivalis Detection by Real-Time PCR

Real-time PCR was conducted for 40 cycles, and the cycles at which *P. gingivalis* and mouse GAPDH DNA were detected were recorded. Samples in which *P. gingivalis* was not detected after the complete on of 40 cycles were given a value of 40. Using these parameters, mouse GAPDH was detected in 14 of 15 samples in the infection group, with *P. gingivalis* detected in 11 samples. The mean cycle threshold (C^T) value for the mouse GAPDH was 14.97, whereas the mean C^T value for *P. gingivalis* was 35.26. Mouse GAPDH was detected in 14 of 15 samples in the QSI group, whereas *P. gingivalis* was detected in eight samples. The mean C^T value for the mouse GAPDH was 15.18, whereas the mean C^T value for *P. gingivalis* was 37.15 (Table 1, Figure 3A and Figure 3B).

The difference in the C^T values for *P. gingivalis* and mouse GAPDH (*P. gingivalis* C^T − mouse GAPDH C^T) was calculated for each sample. The mean ΔC^T values were −20.30 and −21.96 for the infection and QSI groups, respectively. When the ΔC^T value of the infection group was corrected to 0 ($\Delta\Delta C^T$: ΔC^T of the infection group − ΔC^T of the infection group) for normalization, the $\Delta\Delta C^T$ of the QSI group (ΔC^T of the QSIs group − ΔC^T of the infection group) was 1.66. Also, when the $2^{-\Delta\Delta CT}$ method was used to compare the groups, the QSI group exhibited

a reduced bacteria DNA amount of *P. gingivalis* (31.64%) compared with the infection group (Livak and Schmittgen 2001).

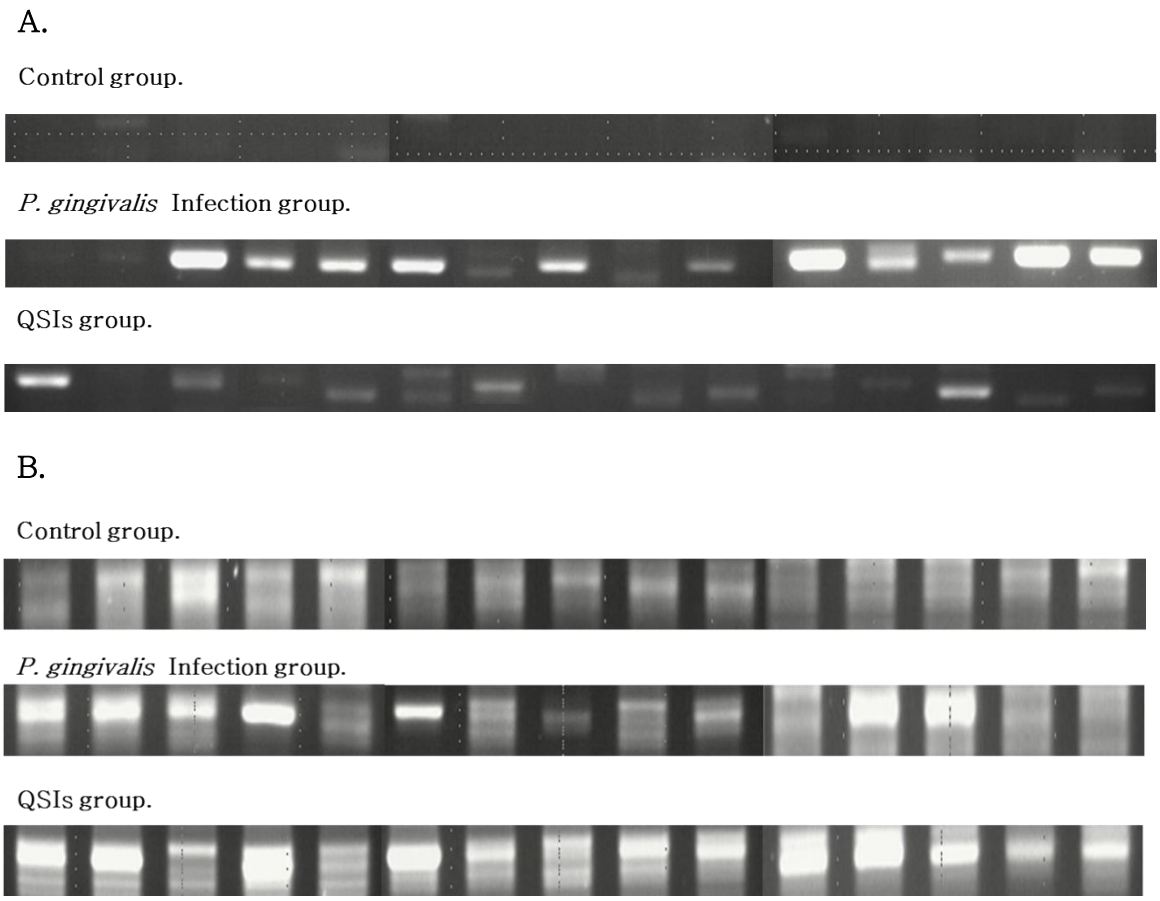

Figure 3.

A. Real-Time PCR for detection of *P.gingivalis* in samples

B. Real-Time PCR for detection of Universal primer (mouse GAPDH DNA)

Table 1. Summary of the estimated ABC – CEJ distances in the M1–M2 interproximal area (mm)

Group	Sample size	Mean (SD)	95% CI		Min	Max
			Lower	Upper		
Control group	15	0.1232 (0.1486)	0.1165	0.13	0.100 4	0.142 9
Infection group	15	0.1438 (0.7872)	0.1279	0.1596	0.1059	0.1911
QSIs group	15	0.122.5 (0.2937)	0.113	0.132	0.886	0.154

Abbreviations: ABC, alveolar bone crest; CEJ, cemento–enamel junction; M1, maxillary first molar; M2, maxillary second molar; QSIs, quorum sensing inhibitors

Micro-CT Analyses of the Interproximal Area

Table 2 and Figure 4 summarize the distributions of bone level after the 6-week period. The mean distance from the CEJ to the ABC in the infection group was significantly longer than that of the control (CMC only) group (mean ± SE of difference: 0.0205 ± 0.0080 mm, P = 0.02), which confirmed that the *P. gingivalis* infection breakdown model used in the current study successfully shows breakdown of alveolar bone as expected. In addition, the mean distance from the CEJ to the ABC in the QSI group was significantly shorter than that of the infection group (mean ± SE of difference: 0.0212 ± 0.0086 mm, P = 0.02), suggesting that the QSIs successfully prevented alveolar bone loss (ABL) from *P. gingivalis* infection.

Furthermore, the 95% confidence interval (CI) of the difference of mean distances from the CEJ to the ABC between the QSI and control group was −0.0102 to 0.0117 mm, which satisfied the predefined equivalence margin of −0.06 to 0.06 mm, demonstrating that the degree of the protection effect was as good as the negative control. 3D image reproductions are shown in Figure 5.

Table 2. The relative comparison of *P. gingivalis* level in the QSIs group relative to the infection group.

Note) Aliquots of cDNA were used as templates for real-time PCR reactions containing either primers and probe for *P. gingivalis* or primers and probe for GAPDH. Each reaction contained cDNA derived from 100 ng total DNA.

Group	*P. gingivalis* C^T	GAPDH C^T	*P. gingivalis* amount relative to the infection group
Infection group	35.26	14.97	1.0
QSIs group	37.15	15.18	0.3164

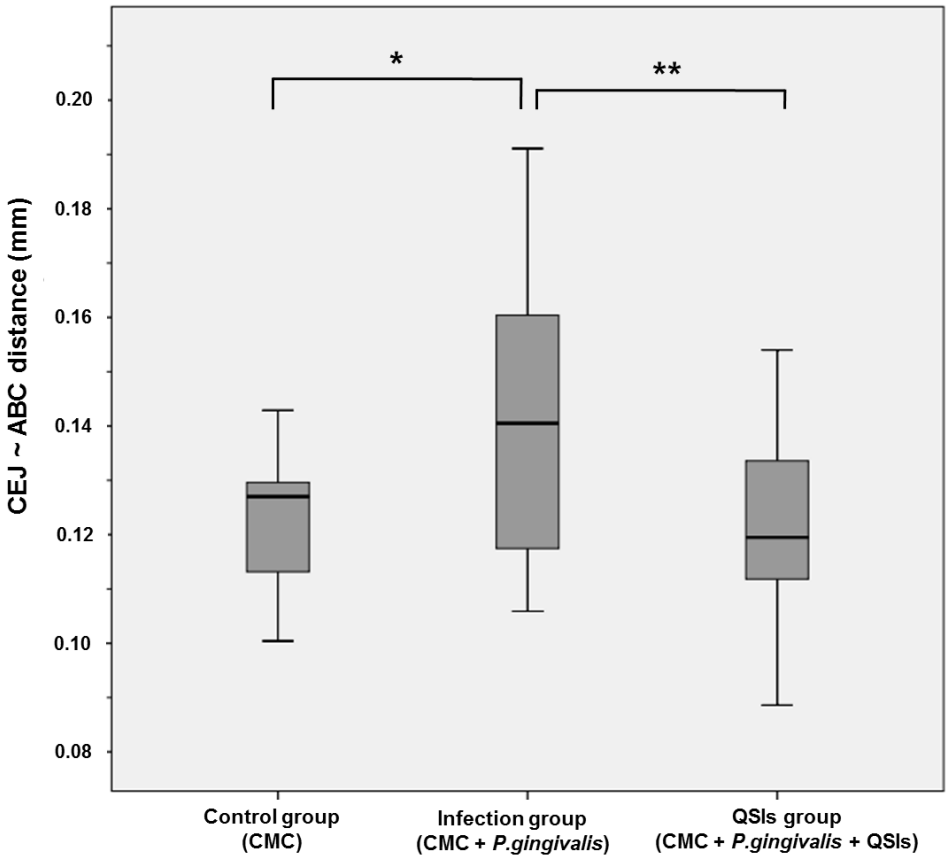

Figure 4. Histogram showing the CEJ ~ ABC distance on the micro CT results.

The distance from the CEJ to ABC of infection group was significantly higher than the control group. And the distance of QSIs group was significantly lower than infection group.

In addition, there was no signification with control group and QSIs group

*, P = 0.016 (significant difference) compared with the control group.

**, P = 0.020 (significant difference) compared with the QSIs group.

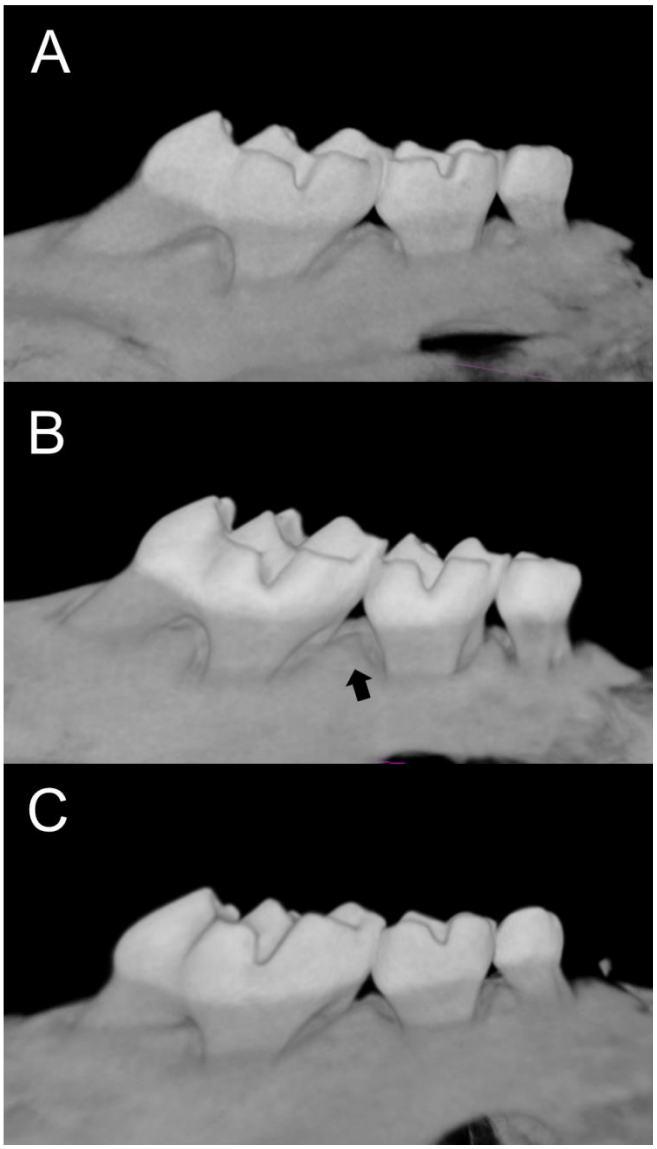

Figure 5. Three dimensional micro CT images at 7 weeks after infection.

(A) Control group, (B) Infection group, (C) QSIs group.

In infection group, significant bone loss was observed at proximal and marginal area of the premolar. Otherwise, there was no significant difference between the QSIs group and the control group.

IV. DISCUSSION

To the best of the authors' knowledge, this *in vivo* study is the first to evaluate the effectiveness of two different AI-2 QSIs: 1) a D-ribose, and 2) a furanone compound. D-ribose and the furanone compound have been known as representative compounds of the AI-2/LuxS QS inhibition system. The AI-2/LuxS QS system is the most conserved signaling pathway for both Gram-positive and Gram-negative bacteria (Hardie and Heurlier 2008). *P. gingivalis* also uses the AI-2 system for signaling and biofilm attachment, and expression of virulence for *P. gingivalis* can be controlled by the LuxS gene (Chung, Park et al. 2001, Burgess, Kirke et al. 2002). Recently, several QSI *in vivo* studies have reported results concerning subcutaneous infection (Cirioni, Mocchegiani et al. 2013), *Pseudomonas aeruginosa* infection (Christensen, van Gennip et al. 2012), and *Staphylococcus epidermidis* infection (Balaban, Giacometti et al. 2003). Although combined effects of antibiotics and QSIs were evaluated, the focus was on the action of QSIs alone for regulation of periodontitis and biofilm growth inhibition.

To reproduce an inflammation around the periodontal tissue, a modified mouse infection model was used (Baker, Evans et al. 1994). This model has been widely used to reproduce experimental periodontitis in rodents and is capable of inducing significant bone loss (Baker, Evans et al. 1994, Lalla, Lamster et al. 2003, Tani-Ishii, Minamida et al. 2003, Polak,

Wilensky et al. 2009, Shusterman, Durrant et al. 2013). After *P. gingivalis* inoculation, the bacteria of each group were expected to establish a mature biofilm that binds irreversibly to the surface of the pellicle layer, not to planktonic cells. To compare the groups using real-time PCR, the tooth and the surrounding soft tissue were harvested and mixed to acquire a mature biofilm. Real-time PCR and the $2^{-\Delta\Delta CT}$ methods were used for the relative comparison of biofilm load among the groups. The QSI group exhibited only 31.6% of the *P. gingivalis* DNA count compared with the infection group. It indicates that the topical use of the two QSIs reduced the amount of biofilm states load.

The natural compound (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone is known to inhibit the pathway of AI-2 and AI-1 and interacts with LuxS gene in the AI-2 QS system without affecting their growth of bacteria (Ren, Sims et al. 2001, Pan and Ren 2009). The synthetic furanone compound that is used in the current study interacts with the LuxS protein in the AI-2QS system and has been shown to inhibit the biofilm formation of *P. gingivalis* in vitro (Pan and Ren 2009, Jang, Choi et al. 2013). The LuxS gene of *P. gingivalis* is required for synthesizing AI-2, and mutation of LuxS gene diminished AI-2 production in P.gingivalis (Burgess, Kirke et al. 2002).

D-Ribose also competed with AI-2-interacting protein RbsB or LsrB, and the biofilm growth by *Aggregatibacter actinomycetemcomitans* was reduced in the presence of D-ribose (Shao, Lamont et al. 2007). Similar

to previous studies, QSI used for drinking water and topical injection in this study can interact with LuxS gene of *P. gingivalis* and induce *P. gingivalis* to be washed out on planktonic state by self-purification. Unlike antibiotics, QSIs do not affect the survival of bacteria.

From the micro-CT results, the bone breakdown of the infection group was significantly higher than the control group that received only the CMC. This suggests that inoculation of *P. gingivalis* leads to a high bacterial cell density, and the cysteine proteases Arg-gingipain and Lys-gingipain are released by *P. gingivalis*. These are two major cysteine proteinases by *P. gingivalis* associated with LuxS protein and interact with adhesion and periodontal tissue damage (Burgess, Kirke et al. 2002). When the QSI group was compared with the infection group, the distance between the ABC and CEJ in the QSI group was significantly lower than the infection group. This finding suggests that D-ribose and furanone compound disrupt AI-2 biosynthesis of *P. gingivalis*. It inhibits LuxS gene expression, eventually suppressing periodontal disease.

These results are consistent with some previous in vitro studies (Jang, Choi et al. 2013, Jang, Sim et al. 2013). This may be a meaningful approach for reducing the progression of periodontitis. Because QSIs use a different mechanism to prevent bacterial growth from antibiotics, the use of QSIs can prevent the antibiotic resistance. When furanone and D-ribose were used against *F. nucleatum* AI-2, one previous study (Jang, Choi et al. 2013) that only examined single-species biofilm growth in

vitro also observed a marked decrease in biofilm growth, but this study aims to evaluate the actions of QSIs on multispecies biofilm growth *in vivo*.

For statistical analysis, three sequential hierarchical statistical tests were performed as follows to control the familywise error rate to a level of 0.05. To validate a successful breakdown of the alveolar bone in the *P. gingivalis* infection model used in the current experiment, the mean ABC–CEJ distance in the infection group was compared with that of the control group. If the mean distance was significantly longer in the infection group than the control group, the mean distance in the QSI group was compared with that in the infection group to prove the beneficial effect of QSIs on the protection of ABL. If the mean distance was significantly shorter in the QSI group than the infection group, an equivalence test to compare the mean distances from the CEJ to the ABC between the QSI and control group was conducted to demonstrate that the degree of the inhibition effect was as good as the control group. According to the general statistical methods (Walker and Nowacki 2011), the equivalence margin was defined as half of the lower limit of the CI of the difference between the control and the infection group from a previous study that used the same breakdown model (Baker 2005). From the literature in detail, the difference of the means between the control and infection groups was 0.32 mm, and their standard deviation (SEs) were 0.7 mm. Based on the independence of the two groups, the SE of the difference of the means between the two groups can be calculated as 0.10 mm, or square root of

the sum of square of 0.7 mm. Thus, the 95% CI of the difference of the means between the control and infection group was estimated at 0.12 to 0.52 mm. Finally, as defined above, the equivalence margin was defined as half of the lower limit of the CI, or 0.06 mm.

In the oral cavity, a rich source of microorganisms and their dynamic interaction exist (Aas, Paster et al. 2005). but in this study, antibiotics are administered to kill the commensal bacteria focusing only on *P. gingivalis* biofilm growth. The interactions between other bacteria, including *F. nucleatum* and *T. forthysia* were not observed.

This is a pilot study testing a new QSI compound, and future studies will aim to optimize the concentrations and the applied frequencies or volume of QSIs and should focus on combining effects with antibiotics to the treatment of human periodontitis.

The real-time PCR was used for the relative comparison of bacterial counts between the infection and QSI groups. As a result, the DNA amount of bacteria in the QSI group was only 31.6% of the infection group. It supports that the QSIs interfere with the LuxS/AI-2 QS system and biofilm formation *in vivo*, and it further supports that QSIs are a new approach to the prevention and treatment of periodontitis.

V. CONCLUSIONS

The observations from the present study suggest that the regular application of D-ribose in the drinking water and the topical inoculation of furanone significantly interfere with biofilm growth. QSIs are expected to play an important role to interfere with the virulence expression of *P. gingivalis*.

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국문 초록

쿼럼 센싱 억제제를 이용한 *Porphyromonas gingivalis* 성장 억제 및 치주질환 예방에 대한 *in vivo* 연구

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1. 연구 목적

Autoinducer (AI)-2는 구강 내에서 바이오 필름 형성에 매우 중요한 역할을 한다. AI-2를 이용한 세포간 신호전달은 바이오 필름을 성숙되게 하고, 이런 성숙한 바이오 필름은 치주염을 치료하기 위한 항생제의 침투를 어렵게 한다. AI-2와 경쟁하는 quorum-sensing 억제제 (QSIs)에 대한 연구는 이미 존재하였으나 생체 외 연구만 존재했다. 이 연구는 실험동물 모델에서 *Porphyromonas gingivalis*의 구강 투여를 통하여 치주염을 유발하고 QSIs을 사용하여 억제력을 비교하는 실험이다.

2. 연구 방법

45 마리의 male mice를 각각 15마리씩 무작위로 1) *P. gingivalis* 감염군, 2) QSIs 적용군, 3) 대조군 3개의 그룹으로 나누었다. *P. gingivalis* 감염군과 QSIs 적용군은 *P. gingivalis*를 구강 내에 직접 적용하였으며, QSIs그룹은 QSIs 물질 (furanone compound 와 D-ribose) 를 구강 내 직접 적용하고, 매일 음용수에 섞어 복용하였다. 대조군은 음성 대조군의 역할을 위하여 구강 내 도포를 위한 용매만 적용하였다. 42일 후 쥐를 희생하였으며, micro CT를 이용하여 alveolar bone crest (ABC) 부터 cemento-enamel junction (CEJ)까지의 거리를 측정하였다. 또한 치아 및 치아 주변 연조직을 채취하여 real-time polymerase chain reaction을 시행했고 이를 통하여 *P. gingivalis* DNA 를 정량적 비교하고자 하였다.

3. 결과

ABC부터 CEJ까지 거리는 *P. gingivalis* 감염군과 대조군의 비교에서 *P. gingivalis* 감염군에서 유의미하게 증가하였다. (P = 0.02) 또한 *P. gingivalis* 감염군과 QSIs 적용군의 비교에서는 QSIs 적용군의 거리가 유의미하게 감소하는 것을 관찰할 수 있었다. (P = 0.02). Real-time PCR의 결과 QSIs 적용군의 *P. gingivalis* DNA count 가 *P. gingivalis* 감염군에 비해 31.64%로 감소하였다.

4. 결론

쥐 모델에서 QSIs의 임상적 사용은 생체 내 상황에서 치주염에 의한 골 소실의 감소를 나타내었으며, 콜로니 형성을 억제하여 치아 주변 바이오 필름 내의 세균 DNA 양의 감소를 보였다.

이는 QSIs가 치주염을 새로운 기전을 통해 예방하고 극복할 수 있으며, 항생제나 기존 치료방법을 대체하는 시도로 받아들여질 수 있음을 의미한다.

주요어: 바이오 필름; 동물 모델; *Porphyromonas gingivalis*; 쿼럼 센싱 ; ribose

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