Inhibition of Cyclosporin A Induced Gingival Overgrowth by Azithromycin through Phagocytosis : An In vivo and In vitro Study

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ABSTRACT

Inhibition of Cyclosporin A Induced Gingival Overgrowth by Azithromycin through Phagocytosis: An In vivo and In vitro Study

Background: The objective of the present study was to investigate the effect of cyclosporin A (CsA) and azithromycin (AZI) on collagen metabolism in the gingiva of rats.

Methods: Fifty 6-week-old male Sprague-Dawley(SD) rats(weight 120 to 150g) were randomly distributed into five groups. All groups received various drugs via gastric feeding for 7weeks. The first group(Mo group) received mineral oil for 7weeks as a control; the CsA group received CsA in mineral oil for 7weeks(dosage 30mg/kg); the CsA/Mo group received CsA in mineral oil for 6weeks and mineral oil only for the seventh week; the CsA/AZI group received CsA in mineral oil for 6weeks and AZI(dosage 10mg/kg) in mineral

oil simultaneously with CsA in the seventh week; and the Mo/AZI group received mineral oil for 6weeks and AZI in mineral oil for the seventh week. All animals were sacrificed for clinical and histological analyses. Gingival fibroblasts were cultured at the fourth passage, and the amount of collagen was measured. Type I collagen and collagenase mRNA were measured by reverse transcription-polymerase chain reaction. Collagen phagocytosis assay also was performed.

Results: Clinically, CsA induced gingival overgrowth in rats, whereas AZI reduced gingival overgrowth. Histological results of the CsA group showed a marked increase of tissue volume compared to the other groups. High collagen amounts were found when gingival overgrowth was induced. However, type I collagen mRNA and collagenase mRNA expressions did not statistically differ among groups. Phagocytosis assay showed that CsA decreased phagocytic activity of gingival fibroblasts, whereas AZI increased the activity. These

results suggest that the induction and reduction of CsA-induced gingival overgrowth were closely associated with phagocytic activity.

Conclusion: Cyclosporin A decreases collagen degradation by lowering phagocytic activity of rat gingival fibroblasts. Azithromycin partially compensates for this lowered phagocytic activity.

KEY WORDS: Cyclosporin A, Azithromycin, Collagen metabolism, Phagocytosis, Reverse transcription-polymerase chain reaction.

Inhibition of Cyclosporin A Induced Gingival Overgrowth by Azithromycin through Phagocytosis : An In vivo and In vitro Study

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Ⅰ.INTRODUCTION

Cyclosporin A(CsA) has been widely used to prevent organ transplant rejection and to treat various immuno-diseases[3,6,37] due to its suppressive effect on specific T lymphocytes and inhibition of certain lymphokines.[4,5] Despite the

effectiveness of CsA, its clinical application is limited due to various side effects such as cardiotoxicity, nephrotoxicity, and gingival overgrowth.[13,34,36,43]

Previous reports showed that this gingival overgrowth was due to accumulation of extracellular collagenous component.[2] Collagen is precisely balanced by collagen synthesis and degradation to maintain a steady state.[35] Thus, gingival overgrowth is a manifestation of the loss of homeostasis, resulting in a surplus of matrix proteins, particularly collagen.[25] There are two pathways of collagen degradation: one is the extracellular pathway involving secretion of collagenase, and the other is the intracellular pathway involving phagocytosis.[8,25,27,35] Normal collagen turnover is primarily controlled by the collagenase-independent route(intracellular pathway), while the collagenase-mediated route(extracellular pathway) predominates during inflammation. Despite many investigations, the etiology of CsA-induced gingival overgrowth[1,12,17,18,32,28] remains unknown.

Clinically, Azithromycin(AZI) has been reported to reduce drug-induced gingival overgrowth.[7,24,28,41,42] AZI is a macrolide antibiotic of the azalide subclass with a long half-life that suppresses protein synthesis of both Gram-positive and Gram-negative aerobes. It has been reported that CsA-induced gingival overgrowth can be effectively treated with a 5-day course of AZI while maintaining CsA therapy.[26,30,39] However, the mechanism of this inhibitory effect has not been identified.

Fu et al. established a Sprague-Dawley(SD) rat model of CsA-induced gingival overgrowth in which macroscopic overgrowth can be observed as early as day 15 after first administering CsA.[9-11] We used this rat experimental model to eliminate major variables such as inflammation, which allowed us to better clarify the etiology of CsA-induced gingival overgrowth.

In the present study, we induced gingival overgrowth by CsA and then reduced the overgrowth by cessation of CsA or administration of AZI in SD

rats in vivo. We also measured the amount of collagen in vitro. The objective was to investigate the effect of CsA and AZI on collagen metabolism in the gingiva of rats.

Ⅱ. MATERIALS AND METHODS

Experimental Design

Fifty 6-week-old male Sprague-Dawley rats(weight 120 to 150g) were randomly distributed into five groups. The Mo group received mineral oil(1ml) daily via gastric feeding for 7weeks as a control. The CsA group received CsA[*](dosage 30mg/kg) in mineral oil via gastric feeding for 7weeks. The CsA/Mo group received CsA(dosage 30mg/kg) in mineral oil daily via gastric feeding for 6weeks, and mineral oil only for the seventh week. The CsA/AZI group received CsA(dosage 30mg/kg) in mineral oil daily via gastric feeding for 6weeks and AZI[†](dosage 10mg/kg) in mineral oil simultaneously with CsA in the seventh week. The Mo/AZI group received mineral oil for ----------

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[*] Sandimmun Neoral, Novartis, Basel, Switzerland.

[†] Zithromax, Pfizer, Seoul, Korea.

6weeks, and AZI(dosage 10mg/kg) in mineral oil for the seventh week(Figure 1). Rats were weighed daily, and the drug amounts were adjusted to maintain constant dosage. The animals were maintained in plastic cages in a room with 12-hour day/night cycles and an ambient temperature of 25°C, and allowed free access to water *ad libitum* and standard laboratory pellets. Animal selection and management followed protocols approved by the Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea.

Clinical Analysis

To record the sequence of gingival changes in the mandibular anterior region, alginate impressions under general anesthesia, with an intramuscular injection(5mg/kg body weight) consisting of ketamine hydrochloride[‡] and

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[‡] Ketalar, Yuhan Co., Seoul, Korea.

stone casts, were made every 2weeks. The mesiodistal width, labio-lingual width, and vertical height at the interdental papilla level and the keratinized gingiva level in the mandibular anterior segment were measured according to the method of Fu et al.[11] on the stone cast with a stereomicroscope[§](Figure 2).

Histological Analysis

Rats were sacrificed by using CO_2 gas at the end of the experiment, and the jaws were immediately dissected and fixed in 10% neutral formalin. Following decalcification in 5% hydrochloric acid, 3μm serial sections were made on the labio-lingual aspects of the central incisor area. Routine hematoxylin-eosin staining was performed as described previously,[23] and light microscopic examination[§] was performed.

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§ Olympus, Olympus Co., Tokyo, Japan.

Gingival Fibroblast Culture

Rat gingival fibroblasts from gingival biopsies were seeded into T-25 flasks‖ at fourth passage and grown in alpha-minimal essential medium(α-MEM) ¶ supplemented with 10% fetal bovine serum(FBS) ¶ and antimycotic-antibiotic solution.[20]

Collagen assay

The amount of collagen in the five groups was assessed using a commercially available kit. # Briefly, triplicate cultures of confluent cells in 96-well plates(1×10^4cells/well) were incubated in the presence of 5% FBS. Test samples(100μl) from each well were added to 100μl dye reagent in 1.5μl tubes.

‖ Nalge, Nunc International, Rochester, NY.

¶ Gibco BRL Life Technologies, Gaithersburg, MD.

[#] Sircol collagen assay kit, Biocolor Ltd., Galway, Ireland.

The tubes were mixed gently at room temperature for 30minutes and then centrifuged for 10minutes at 10,000rpm. The supernatant was drained off, and 100μl of the alkali reagent was added. Samples were assessed under 530nm wavelength using an enzyme-linked immuno-sorbent assay reader. [**]

Reverse Transcription-Polymerase Chain Reaction(RT-PCR) for Type I Collagen and Collagenase

Gingival fibroblasts were seeded onto 60mm dishes at a density of 2 × 10^5cells/dish. At confluence, total RNA was extracted using a reagent[††] according to the manufacturer's instructions. The concentration of the RNA was determined by spectrophotometry measuring optical density(OD) at 260

[**] DYNATECH Lab., Chantilly, VA.

[††] TRIZOL, Gibco BRL Life Technologies.

and 280nm. RT-PCR for type I collagen and collagenase mRNA was carried out with a commercial RNA PCR kit.[‡‡] Total RNA (1μg) isolated from each sample was used as a template for cDNA synthesis. Reverse transcription of total RNA was performed for 15minutes at 42°C in a reaction buffer containing 1mM dNTP mixture, 1μM antisense primer, and 0.25U/μl reverse transcriptase. The cDNA was used immediately or stored at –20°C until used. The cDNA was amplified by PCR in 80μl reaction mixture containing 20μl of each cDNA. The reaction mixture contained 2.5U/100μl of *Taq* polymerase,[‡‡] 1X reaction buffer and 0.2μM of sense primer mix. PCR was performed for 30cycles of 94°C for 1minute, 60°C for 1minute, 72°C for 1minute, and the tubes cooled to 4°C in a thermocycler. [§§] PCR conditions and the sequences of the primers used are shown in Table 1. ----------

----------[‡‡] Takara RNA PCR kit, Takara Shuzo Co., Ltd., Shiga, Japan.

[§§] T gradient, Biometra, Göttingen, Germany.

Table 1. PCR oligonucleotide primers

Primers	Sequence	Size (bp)	A.T. (°C)	Thermal cycles
Collagen[18]	Sense 5' AACGATGGTGCCAAGGGTGAT 3' Anti 5' ATTCTTGCCAGCAGGACCAAC 3'	1077	60	30
Collagenase[18]	Sense 5' AGTTGGACTCACTGTTGGTCC 3' Anti 5' ACAGTTCAGGCTCAACCTGCT 3'	928	60	30
β-actin[15]	Sense 5' AACCGCGAGAAGATGACCCAGATCATGTTT 3' Anti 5' AGCAGCCGTGGCCATCTCTTGCTCGAAGTC 3'	351	60	30

After amplification, 16μl of each PCR product was analyzed by 1.3% agarose gel electrophoresis; the bands were visualized by ethidium bromide staining; and the gels were photographed using a gel documentation system‖‖ under ultraviolet light.

Phagocytosis Assay Using Flow Cytometer

Phagocytosis assay was measured using 2.0μm fluorescent latex beads¶¶ coated with soluble bovine type I collagen according to a previously described method.[19,21,25] Gingival fibroblasts were seeded onto six-well plates at a density of 4×10^4cells/well. At confluence, fibroblasts were incubated with coated beads(bead:cell ratio, 4:1) for 16hours, and the cells were washed in ---------

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[||||]Gel Doc 1000, Bio-Rad, Hercules, CA.

PBS at 37°C then detached with 0.01% trypsin[¶] at 37°C for 10minutes. They were resuspended in 1ml PBS and kept at 4°C. The trypsin was neutralized by the addition of an equal volume of medium containing 15% FBS, washed in PBS, and fixed for 30minutes at 4°C in 80%(v/v) ethanol added drop-wise to the cells. For flow cytometry, the cells were analyzed at a rate of 200cells/second on a flow cytometer[##] with 488nm excitation and emission measured with a 530/30band pass filter. Populations of phagocytic and non-phagocytic cells were verified by cell sorting. The percentage of phagocytic cells was determined by the formula([number of cells with internalized beads/total number of cells]×100).

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Molecular Probes Inc., Eugene, OR.

[##] FACS Calibur, Becton-Dickinson Immunocytometry System, San Jose, CA.

Statistical Analysis

Statistical differences were determined by analysis of variance and post-hoc *t* test for multiple comparisons. Statistical significance was determined at the $P < 0.05$ level.

Ⅲ. RESULTS

Clinical and Histological Analyses

Gingival overgrowth was observed in the three groups that had received CsA. The overgrowth in the mesiodistal and labio-lingual width significantly increased at the interdental papilla level at 4weeks, while the vertical height increased markedly at the keratinized gingiva level at 6weeks. After 6weeks, gingival overgrowth decreased in the CsA/Mo group and CsA/AZI group. These two groups represented similar patterns despite continuous CsA administration in the CsA/AZI group(Table 2 and Table 3).

Histological examination of the CsA group showed marked increase of tissue volume compared to the other groups. There were few inflammatory cells in the overgrown gingiva(Figure 3).

Table 2. Gingival changes at the interdental papilla level

Group	Baseline	2 wks	4 wks	6 wks	7 wks
	Mesiodistal width(㎜)				
Mo	1.36±0.12	1.46±0.26	1.5±0.15	1.53±0.10	1.54±0.09
CsA	1.34±0.11	1.57±0.16	1.99±0.13*	2.08±0.21*	2.20±0.20*
CsA/Mo	1.31±0.13	1.59±0.17	1.83±0.05*	2.14±0.19*	1.62±0.16
CsA/AZI	1.32±0.12	1.58±0.18	1.84±0.05*	2.20±0.13*	1.52±0.11
Mo/AZI	1.30±0.10	1.48±0.13	1.54±0.11	1.54±0.11	1.54±0.09
	Labiolingual width(㎜)				
Mo	1.93±0.13	2.04±0.13	2.26±0.16	2.32±0.18	2.38±0.19
CsA	1.99±0.12	2.26±0.12	2.50±0.21*	2.63±0.09*	2.72±0.27*
CsA/Mo	1.69±0.11	2.23±0.10	2.38±0.13	2.52±0.08*	2.38±0.19
CsA/AZI	1.70±0.09	1.98±0.23	2.27±0.08	2.54±0.09*	2.28±0.16
Mo/AZI	1.76±0.09	1.98±0.13	2.34±0.15	2.36±0.13	2.38±0.15
	Vertical height(㎜)				
Mo	1.26±0.15	1.39±0.14	1.39±0.19	1.45±0.13	1.46±0.13
CsA	1.23±0.08	1.40±0.11	1.53±0.16	1.66±0.23	1.72±0.18*
CsA/Mo	1.10±0.12	1.46±0.05	1.48±0.28	1.77±0.14	1.40±0.14
CsA/AZI	1.15±0.10	1.38±0.12	1.42±0.11	1.62±0.25	1.40±0.07
Mo/AZI	1.18±0.11	1.42±0.08	1.42±0.04	1.46±0.11	1.47±0.12

* Statistically significant differences compared with control group(Mo group)

at $p<0.05$.

Table 3. Gingival changes at the keratinized gingiva level

Group	Baseline	2 wks	4 wks	6 wks	7 wks
	Mesiodistal width(㎜)				
Mo	4.00±0.34	4.54±0.21	4.56±0.18	4.58±0.12	4.60±0.16
CsA	4.04±0.21	4.56±0.17	4.87±0.18	5.00±0.37	5.04±0.22
CsA/Mo	3.80±0.17	4.56±0.19	4.80±0.14	5.04±0.39	4.57±0.30
CsA/AZI	3.91±0.18	4.49±0.14	4.84±0.26	4.92±0.22	4.44±0.11
Mo/AZI	3.96±0.19	4.48±0.16	4.55±0.10	4.56±0.05	4.58±0.16
	Labiolingual width(㎜)				
Mo	3.90±0.24	4.15±0.32	4.22±0.25	4.23±0.24	4.24±0.15
CsA	3.89±0.21	4.16±0.22	4.42±0.21	5.01±0.16*	4.96±0.09*
CsA/Mo	3.51±0.28	4.14±0.26	4.30±0.18	4.80±0.23*	4.44±0.15
CsA/AZI	3.60±0.26	4.08±0.23	4.26±0.17	4.83±0.29*	4.18±0.16
Mo/AZI	3.66±0.25	4.10±0.35	4.15±0.26	4.22±0.30	4.30±0.16
	Vertical height(㎜)				
Mo	4.04±0.24	4.37±0.38	4.48±0.38	4.64±0.30	4.80±0.23
CsA	4.07±0.18	4.68±0.32	4.98±0.21	5.34±0.38*	5.82±0.16*
CsA/Mo	4.03±0.19	4.76±0.13	4.63±0.21	5.56±0.38*	5.12±0.18
CsA/AZI	3.92±0.17	4.72±0.10	4.76±0.09	5.50±0.12*	5.32±0.22
Mo/AZI	3.90±0.20	4.32±0.40	4.53±4.53	4.54±0.36	4.50±0.12

* Statistically significant differences compared with control group(Mo group)

at $p<0.05$.

Collagen Assay

The amount of collagen in the CsA group statistically significantly increased compared to the other groups. The amount of collagen in the CsA/Mo and CsA/AZI groups increased compared to the Mo and Mo/AZI groups(Figure 4). These results were consistent with the clinical observations of gingival overgrowth.

RT-PCR for Type I Collagen and Collagenase

Type I collagen mRNA was detected using RT-PCR; however, there were no significant differences among any of the groups in terms of band density. Collagenase mRNA levels were almost identical(Figure 5). These results

indicated that CsA and AZI have little effect on type I collagen or collagenase mRNA.

Phagocytosis Assay

The percentage of phagocytic cells in the Mo group was 47.78%, and in the Mo/AZI group 51.08%. The Mo/AZI group showed the highest percentage of phagocytic cells, but this was not statistically significant. The percentage of phagocytic cells in the CsA group was 25.78%, which was statistically significantly decreased compared to the other groups. The percentages in the CsA/Mo and CsA/AZI group were 37.39% and 37.04%, respectively. These two groups showed nearly the same values; they also showed a decreased percentage of phagocytic cells, although it was still significantly increased compared to the CsA group(Figure 6). These results showed that CsA decreased phagocytic activity of gingival fibroblasts, whereas AZI increased the activity.

Ⅳ. DISCUSSION

CsA is known to cause gingival overgrowth due to changes in collagen metabolism.[13,34,36,43] Collagen metabolism is precisely regulated by homeostatic balance between collagen synthesis and degradation.[35] However, it is not known exactly what effect CsA has on collagen metabolism.[16,29,33,40]

There are some difficulties in investigating the etiology of CsA-induced gingival overgrowth in humans. The genetic capacity of the host to metabolize administered drugs, the responsiveness of gingival tissue to the drugs, and the preexisting gingival condition may differ among individuals.[29] Therefore, many investigators have attempted to develop a controlled animal model of CsA-induced gingival overgrowth. The Sprague-Dawley rat model used in the

present study is suitable for investigating the etiology of CsA-induced gingival overgrowth. These rats, although they were not inbred, respond quite uniformly to CsA.[31] Humans, on the other hand, respond differently to similar treatment protocols.[6,13,36] In their review article on rat model studies, Nishikawa et al. reported that the incidence of macroscopic gingival overgrowth was 100% after a relatively short administration of drug.[29] The present results are consistent with this. Preexisting gingival conditions such as inflammation were controlled in this model. This is important because inflammation plays a major aggravating role in the process of gingival overgrowth.[29] In the present study, histological examination showed few inflammatory cells in the groups.

We demonstrated in the present study that collagen accumulation in CsA-induced gingival overgrowth is not caused by increased synthesis of collagen but by decreased degradation, since CsA did not affect the synthesis of type I collagen at the transcription level. Specifically, CsA decreased collagen

degradation through an extracellular phagocytosis-mediated pathway, which may be an important event in the normal turnover of collagen.[1,17,35]

In addition, cessation of CsA or administration of AZI resulted in significant inhibition of CsA-induced gingival overgrowth. These results are in agreement with previous clinical reports showing that AZI can treat CsA-induced gingival overgrowth effectively despite continuous administration of CsA.[7,24,26,28,30,39,41,42]

Clinically, AZI has been reported to be highly effective in treating CsA-induced gingival overgrowth.[7,24,26,28,30,39,41,42] These results imply that AZI has an inhibitory effect on CSA-induced gingival overgrowth. However, there are a few reports concerning the inhibitory mechanism of AZI.[14,22,41] Three possible mechanisms could be inferred, the first of which is the inherent drug effect of AZI. Citterio et al. suggested that the effects of AZI on gingival overgrowth might be related to its antibiotic activity, killing oral bacteria, reducing local

inflammation, and suppressing protein synthesis in fibroblasts.[7] The reason why only AZI affects CsA-induced gingival overgrowth needs to be clarified. In the present study, AZI alone had no effect on gingiva or gingival fibroblasts; only when CsA was being administered did AZI decrease gingival overgrowth and increase phagocytic activity of gingival fibroblasts. These findings suggest that there might be an interaction between CsA and AZI. The second possibility is that AZI inhibits the inherent effects of CsA. Some researchers reported the upregulating effect of AZI on the serum levels of CsA,[22] while others reported no effects.[14] The interaction between AZI and CsA is controversial, but no studies could be found to support this second mechanism. The third possibility is that AZI is associated with the inhibition of phagocytosis induced by CsA. This possibility corresponds to the present results, in which AZI significantly increased the lowered phagocytic activity induced by CsA.

CsA is known to be a Ca^{2+} antagonist. It is noteworthy that CsA, phenytoin, and nifedipine, the three drugs that induce gingival overgrowth, are all Ca^{2+} antagonists. These findings suggest that gingival overgrowth may arise from a common pathogenetic basis in association with Ca^{2+}. Kataoka et al. proposed that CsA may block the influx of Ca^{2+}, thus inhibiting phagocytosis, which clinically results in gingival overgrowth.[17] This suggestion has been supported by several articles.[1,18,38] Further studies concerning the changes in the influx of Ca^{2+} are necessary to understand the regulatory mechanisms of AZI on CsA-induced gingival overgrowth.

In summary, CsA-induced gingival overgrowth is a condition of collagen accumulation that can be treated by AZI therapy. The collagen accumulation is not caused by increased synthesis of collagen but rather by decreased degradation. Based on the present results, phagocytosis is thought to be the principal pathway of collagen degradation in CsA-induced gingival overgrowth.

Moreover, treatment with AZI appears to restore part of the phagocytosis mechanism.

In conclusion, our results suggest that cyclosporin A decreases collagen degradation by lowering phagocytic activity of rat gingival fibroblasts, and that AZI partially compensates for this lowered phagocytic activity.

Ⅴ. CONCLUSION

Cyclosporin A decreases collagen degradation by lowering phagocytic activity of rat gingival fibroblasts. Azithromycin partially compensates for this lowered phagocytic activity.

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FIGURE LEGENDS

Figure 1. Study design during 7-week experiment.

CsA and AZI were diluted in mineral oil.

Figure 2. Clinical measurement of gingival changes.

The measurements of mesiodistal width(MD), labio-lingual width(LL), and vertical height(VH) of the interdental papilla level(i) and keratinized gingiva level(k) in the region of the mandibular incisors(This figure is reprinted with permission from Quintessence Publishing Co., Inc.).

Figure 3. Histological views of rat gingival tissue in the mandibular central incisor area. Photomicrographs(×40) of lingual gingiva from 13-week-old Sprague-Dawley rats fed various drugs: **A)** Mo group, **B)** CsA group, **C)** CsA/Mo group, **D)** CsA/AZI group, and **E)** Mo/AZI group.

Figure 4. Amount of collagen in rat gingival fibroblasts.

Collagen amount in the CsA group statistically significantly increased compared to the other groups. *Statistically significant differences compared to the Mo and Mo/AZI groups at $P < 0.05$. †Statistically significant differences compared to the other groups at $P < 0.05$. Experiments were conducted at least three times, and similar results were obtained.

Figure 5. RT-PCR analysis of type I collagen and collagenase mRNAs in the rat gingival fibroblasts.

Note that there are no differences in the band density among any of the groups. Experiments were conducted at least three times, and similar results were obtained.

Figure 6. Flow cytometry of rat gingival fibroblasts with internalized beads.

A) Mo group, **B)** CsA group, **C)** CsA/Mo group, **D)** CsA/AZI group, and **E)**

Mo/AZI group. The CsA group showed a small number of phagocytic fibroblasts compared to the other groups. *Statistically significant differences compared to the Mo and Mo/AZI groups at $P < 0.05$.[†]Statistically significant differences compared to the other groups at $P < 0.05$. Experiments were conducted at least three times, and similar results were obtained.

FIGURES

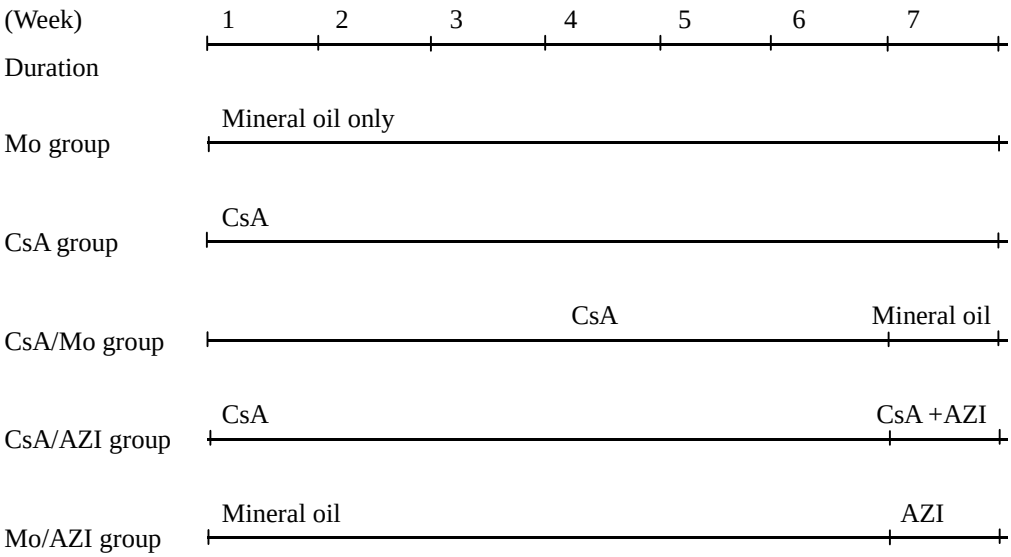

Figure 1. Study design during 7- week experiment

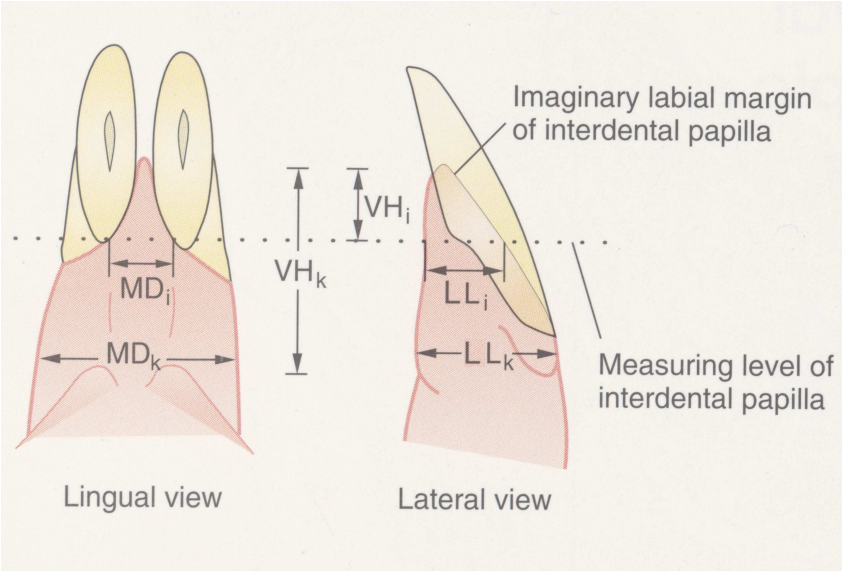

Figure 2. Clinical measurement of gingival changes

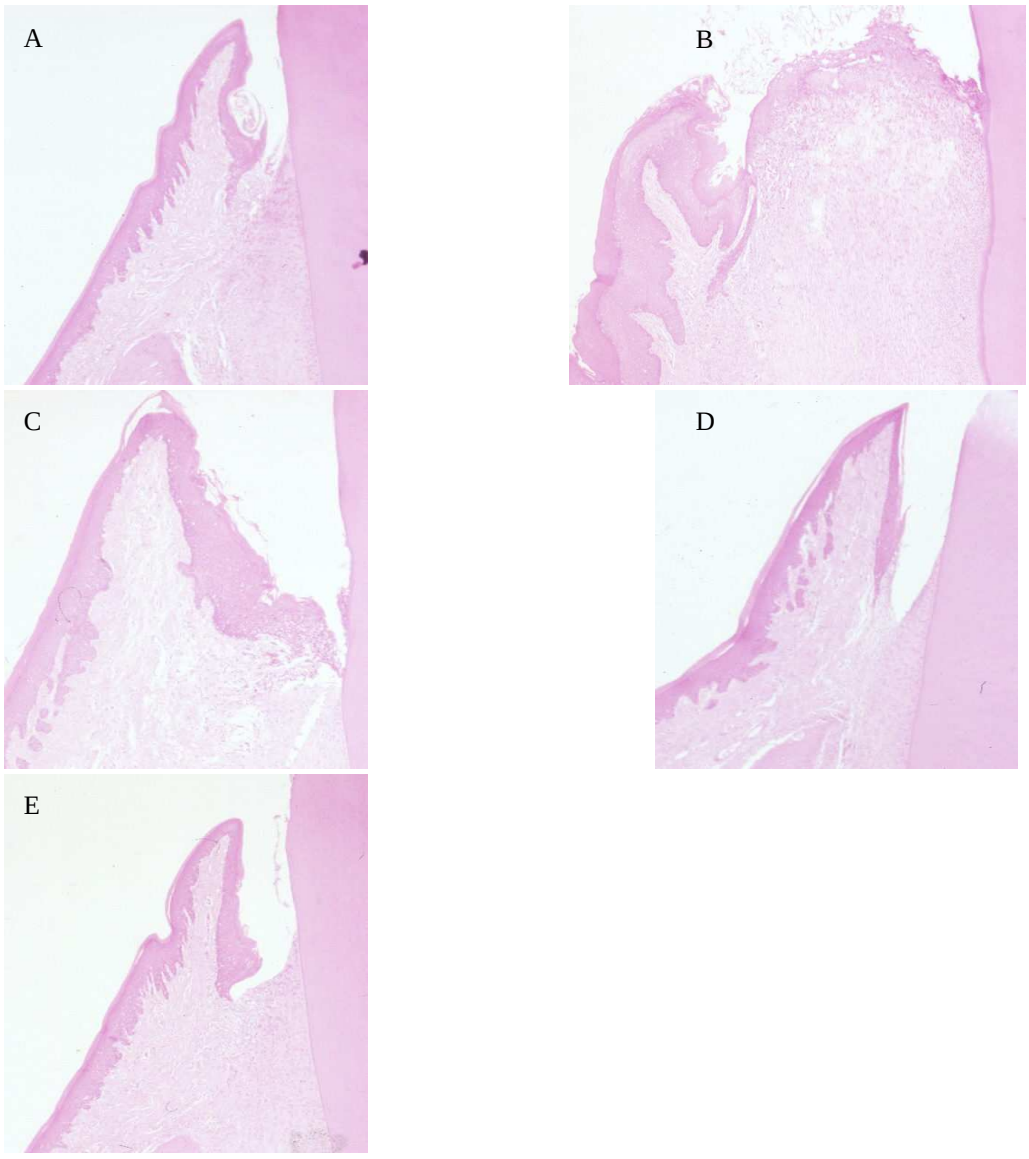

Figure 3. Histological views of rat gingival tissue in the mandibular central incisor area

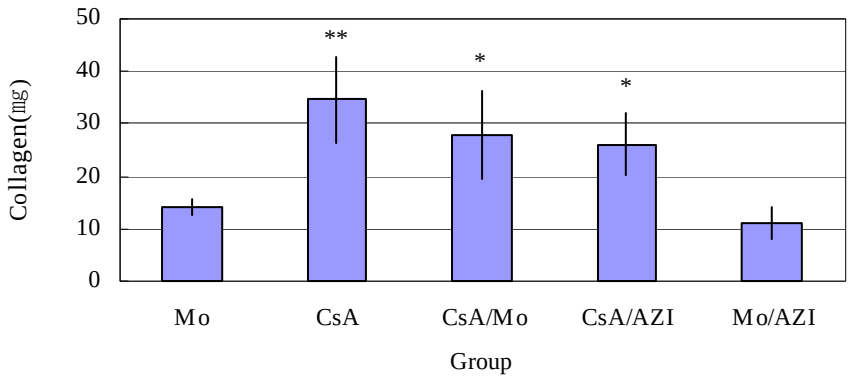

Figure 4. Collagen amount in rat gingival fibroblasts.

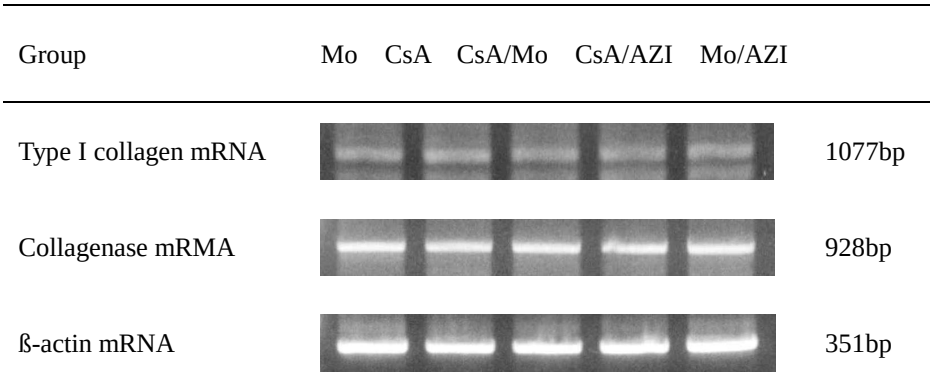

Figure 5. RT-PCR analysis of type I collagen and collagenase mRNAs in the rat gingival fibroblasts.

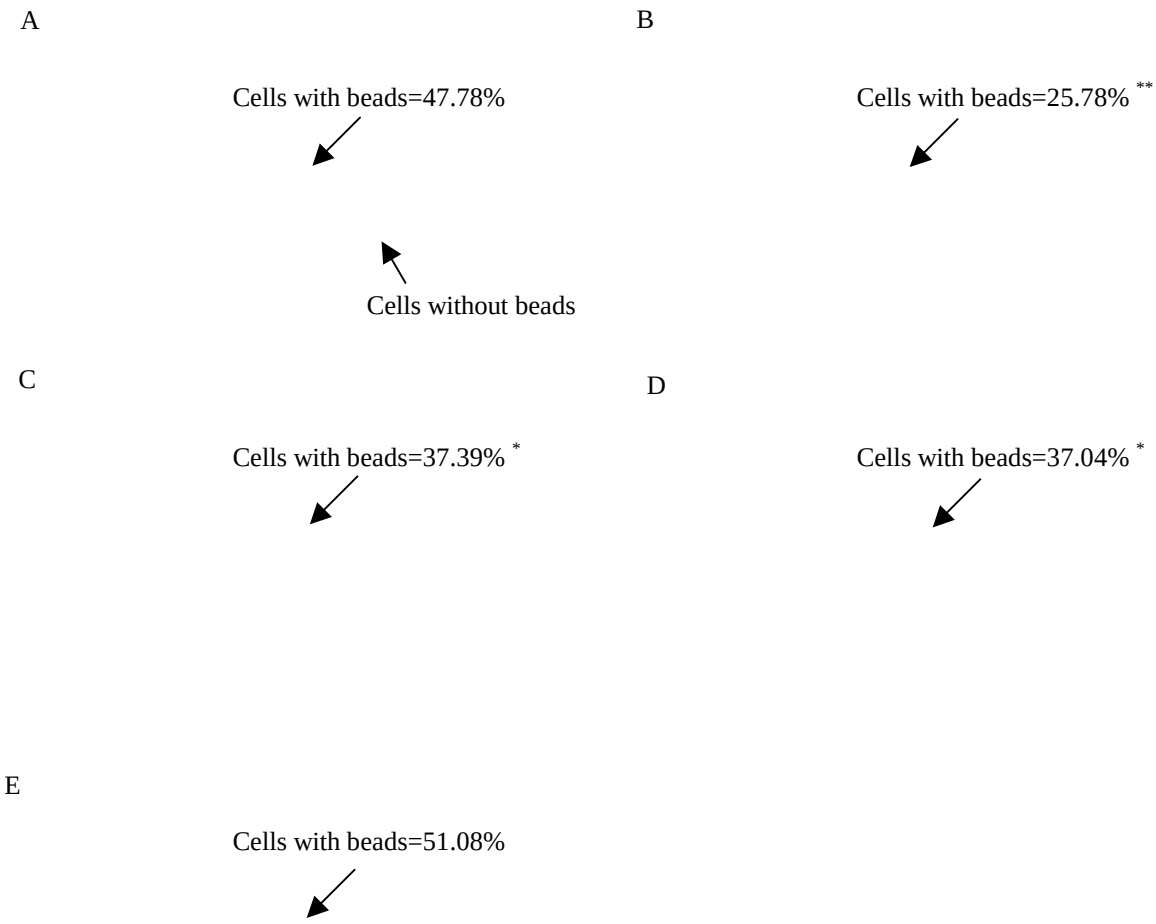

Figure 6. Flow cytometry of rat gingival fibroblasts with internalized beads.

국문 요약

Inhibition of Cyclosporin A Induced Gingival Overgrowth

by Azithromycin through Phagocytosis: An In vivo and In vitro Study

면역 억제제로 널리 사용되는 싸이클로스포린(Cyclosporin A, CsA)은 여러 가지 부작용을 나타내고 있는데 치과와 관련된 부분이 치은 과증식이다. 임상적으로는 아지스로마이신 (Azithromycin)이 이를 감소시킨다는 보고가 있으나, 상기 두 가지 약물이 어떠한 기전으로 치은에 영향을 주는지는 아직까지 명확치 않다. 본 연구의 목적은 백서 치은의 교원질 대사에 미치는 싸이클로스포린과 아지스로마이신의 영향을 알아보고자 함이다. 수컷 백서(Sprague-Dawley Rat, 무게 120-150g) 50마리를 무작위로 5개 군으로 분류하여 7주 간 각기 다른 약물을 위에 투여하였다. 첫 번째 군(Mineral oil 군, Mo 군)은 대조군으로 7주 간 미네랄 오일을 투여하였다; CsA 군은 7주 간 미네랄 오일로 희석한 싸이클로스포린(농도 30㎎/㎏)을 투여하였다; CsA/Mo 군은 6주 간 미네랄 오일로 희석한 싸이클로스포린(농도 30㎎/㎏)을 투여하다가 7주 째는 미네랄 오일만을 투여하였다; CsA/AZI 군은 6주 간 미네랄

오일로 희석한 싸이클로스포린(농도 30㎎/㎏)을 투여하다가 7주 째는 미네랄 오일로 희석한 싸이클로스포린(농도 30㎎/㎏)과 아지스로마이신(농도 10㎎/㎏)을 함께 투여하였다; Mo/AZI 군은 6주 간 미네랄 오일을 투여하다가 7주 째는 미네랄 오일로 희석한 아지스로마이신

(농도 10㎎/㎏)을 투여하였다. 2주 간격으로 모든 군의 하악 전치부 인상을 채득하여 임상적 변화를 계측하였다. 7주 후 모든 군의 동물들은 희생하여 조직학적 검사 및 세포 배양을 시행하였다. 치은 섬유아세포는 4세대까지 배양하여 교원질 양을 측정하였고, I형 교원질과 교원질 분해 효소에 대한 역전사 효소 연쇄 반응(Reverse Transcription Polymerase Chain Reaction, RT-PCR)을 시행하였다. 또한, 교원질 탐식 작용도 분석하였다.

임상적으로, 싸이클로스포린은 백서 치은 과증식을 일으킨 반면, 아지스로마이신은 치은 과증식을 감소시켰다. 조직학적 결과를 볼 때 싸이클로스포린 군은 다른 나머지 군에 비해 현저한 치은 조직의 과증식을 나타내었다. 또한, 치은 과증식이 나타난 군에서는 교원질의 양이 증가되어 있음을 알 수 있었다. 그러나, I형 교원질과 교원질 분해 효소에 대한 전사 리보핵산 발현은 모든 군에서

통계학적으로 차이가 없었다. 탐식 작용 분석에서 싸이클로스포린은 치은 섬유아세포의 탐식 활성을 저하시킨 반면 아지스로마이신은 활성을 증가시켰다. 이러한 결과를 종합할 때 싸이클로스포린 유도 치은 과증식은 탐식 작용과 밀접하게 연관되어 있다고 사료된다.

결론적으로, 싸이클로스포린은 백서 치은 섬유아세포의 탐식 활성을 저하시킴으로써 교원질을 감소시키는 반면, 아지스로마이신은 저하된 탐식 활성을 부분적으로 보상한다.

핵심되는 말: 싸이클로스포린, 아지스로마이신, 교원질, 탐식 작용, 역전사 효소 연쇄 반응