Effects of sub-antimicrobial dose doxycycline therapy on crevicular fluid MMP-8, and gingival tissue MMP-9, TIMP-1 and IL-6 levels in chronic periodontitis

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감사의 글

기나긴 시간, 논문이 나오기까지 끊임없이 격려해 주시고 배려해 주신 김종관 선생님께 깊은 감사를 드립니다. 부족한 제자에게 관심을 잃지 않으시고 도움 주신 문익상 선생님, 채중규 선생님, 조규성 선생님, 최성호 선생님, 최봉규 선생님, 유윤정 선생님께 감사의 말씀을 올립니다.

실험에 많은 도움을 주신 치주과 교실원에게 감사드리고, 항상 옆에서 용기를 주는 아내와 두 딸 예은이 지은이, 멀리 외국에서 격려 해주시는 양가 부모님께 감사드립니다.

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저자 씀

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ABSTRACT

The objective of the present study was to investigate whether sub-antimicrobial dose doxycycline (SDD) therapy for 120 days in chronic adult periodontitis patients had significant effects on gingival crevicular fluid (GCF) matrix metalloproteinase-8 (MMP-8) levels, and on gingival tissue MMP-9, tissue inhibitor of matrix metalloproteinases-1(TIMP-1) and interleukin-6 (IL-6) levels. A total of 32 patients with incipient to moderate (probing pocket depth 4–7 mm) chronic adult periodontitis were included in the study. Subjects were randomly assigned to two groups. After scaling and root planning (SRP), the SRP + SDD group received SDD, 20 mg *bid*, whereas the SRP + placebo group received placebo, 20 mg *bid*. In the follow-up, efficacy measures included the change in probing pocket depth (PD), clinical attachment level (CAL), bleeding on probing (BOP) and gingival crevicular fluid MMP-8 levels, gingival tissue MMP-9, TIMP-1 and IL-6 levels from baseline to 120 d. After 120 days, PD and CAL improved significantly in SRP + SDD group. Initial MMP-8 levels for SRP + SDD group and SRP + placebo group were 407.13 ± 114.45 ng/ml and 378.71 ± 189.39 ng/ml respectively, with no statistical difference between the two groups. MMP-8

levels for SRP + SDD group and SRP + placebo group were: 235.35 ± 134.58 ng/ml and 364.04 ± 219.27 ng/ml at 30 days; 157.50 ± 95.95 ng/ml and 236.60 ± 186.16 ng/ml at 60 days; 102.70 ± 67.64 ng/ml and 208.56 ± 124.54 ng/ml at 90 days; and 63.77 ± 53.33 ng/ml and 229.13 ± 168.09 ng/ml at 120 days, respectively. The amount of decrease in MMP-8 levels for SRP + SDD group was statistically significant compared to that for SRP + placebo group, especially apparent at 120 days (p<0.05). TIMP-1 levels in both groups increased from the baseline to 120 days with statistical significance (p<0.05), but there was no significant difference between the two groups. Changes in MMP-9 and IL-6 levels were not statistically significant. In conclusion, Adjunctive SDD therapy can improve the clinical parameters and this clinical improvement is reflected by controlled level of MMP-8 in chronic adult periodontitis after the therapy.

Key words: sub-antimicrobial dose doxycycline (SDD), MMP-8, MMP-9, TIMP-1, IL-6

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I. INTRODUCTION

Tetracyclines have been used as an effective adjunct to periodontal therapy[1] . Studies have shown that tetracyclines had a unique ability to be concentrated in gingival crevicular fluid (GCF) in relatively higher level compared to serum level, and that they were effective in controlling the gram negative organisms responsible for periodontal diseases[2]. In addition to their use as systemically administered antibiotics, tetracyclines have been used as an effective root conditioning agent and a local delivery agent, largely due to its ability to bind to tooth surface and be released slowly[3] .

More recent studies have shown that tetracycline along with its closely related

forms (doxycycline and minocycline) can be used in sub-antimicrobial dosage to control and suppress the progression of periodontal disease[4,5,6]. These studies demonstrated that tetracycline families could significantly inhibit collagenase activity in GCF and in gingival tissue, even in much lower dosage than a traditional antimicrobial dosage used in conventional periodontal therapy. Sub-antimicrobial dose doxycycline (SDD) therapy has been shown to reduce periodontal disease activity without inducing antimicrobial resistance[7] .

Matrix metalloproteinases (MMPs) are enzymes involved in tissue destruction and regeneration. Extracellular matrix degradation during various periodontal disease is mediated by a complex cascade involving both host and microbial-derived proteinases[8]. In this regards, the host-derived MMPs are thought to play a key role, and enhanced activity of these enzymes is a consequence of microbially-induced inflammation in the periodontal tissues. Especially, polymorphonuclear leucocyte (PMN)-derived MMPs (MMP-8, MMP-9) are the main proteinases related to tissue destruction and remodeling events in periodontal diseases [9].

Tissue inhibitor of matrix metalloproteinases (TIMPs) are modulating factors of MMPs activity, and four members of TIMPs have been reported. Among those, TIMP-1 and TIMP-2, which have inhibitory effects on all MMPs, are found in periodontal lesions[10]. TIMP-2 shows strong inhibition against PMN-derived MMPs, while TIMP-1 shows greater inhibition against fibroblast-derived MMPs[11].

And Interleukin (IL)-6 is a cytokine that is found in increased level in GCF of periodontitis patients and is also reported to be closely related to clinical severity of

periodontitis[12] . Thus, studies on regulations of these factors are not only important to clarify pathogenic mechanisms but may also direct, at least in part, the therapeutic strategy.

The objective of the present study was to investigate whether SDD therapy for 120 days in chronic adult periodontitis patients had significant effects on GCF MMP-8 levels, and on gingival tissue MMP-9, TIMP-1 and IL-6 levels.

II. MATERIALS & METHODS

Selection of Human Subjects

Patients with chronic incipient to moderate adult periodontitis treated in the Department of Periodontology, College of Dentistry, Yonsei University took part in the present study. Informed Consent was obtained from all patients entering the experiment. The study was approved by Review Board of Yonsei Dental Hospital, Seoul, Korea. A total of 32 adult human subjects between the ages of 25 and 64 years were randomly assigned to two groups as SRP + SDD group (SDD therapy following scaling and root planning) and SRP + placebo group (placebo following scaling and root planning).

SRP+SDD group consisted of 15 patients and SRP + placebo group consisted of 17 patients. Patients in SRP + SDD group ranged in age of 25-64 with mean age of 43, and patients in SRP + placebo group ranged in age of 30-63 with mean age of 50. SDD group consisted of 8 males and 7 females, while control group consisted of 9 males and 8 females, with no statistical differences in neither male/female ratio nor age distribution between the two groups.

All subjects had no history of systemic disease or antibiotic therapy for 12 months prior to the experiment.

Clin ical therapy and examination

SRP + SDD group received scaling and root planing therapy and 20mg bid SDD medication (Dentistar® , Hana Pharmaceutical Inc. Seoul, Korea), while SRP + placebo group received root planing therapy and placebo (20 mg bid). One sextant was randomly selected from each patient and the site with greatest initial pocket depth was used as test site. Scaling and root planing was done on one sextant per subject, and one specific tooth was chosen within the sextant for collecting gingival crevicular fluid and gingival tissue samples. Placebo and SDD therapy in SRP + SDD and SRP + placebo group were 120 days in duration. Changes in pobing pocket depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP) from baseline to 120 days were measured. Gingival pocket depth measurement was made by one experienced clinician (D.H. Choi) using Marquis color coded probe (diameter 0.5mm) inserted with apical force of 20-30g. Pocket depth was measured to nearest 1mm.

Sample collection

15 patients received 20 mg doxycyline, and 17 patients received placebo tablets bid for 120 days. GCF samples were obtained from deep periodontal pockets (pocket depth ≒ 4-7 mm) before and on day 30, 60, 90 and 120 of drug intake. Prior to collection, the tooth surface was dried with air and kept dry with cotton wool rolls. Three paper points were inserted into the sulcus for 3 min, and then placed in a vial containing 200:l of the enzyme reaction buffer (ER buffer: 50 mM Tris-HCl, 0.2 M NaCl, 5 mM CaCl2, pH 7.5). Adsorbed fluid was eluted from the paper points by

vigorous vortexing the sample vial and centrifuged at 13,000 x g for 10 min at 4°C. Supernatants were collected and stored at -20°C until required.

Gingival tissue samples were obtained from patients before and on day 120 of drug intake. After surgery, excised tissue samples were immediately placed on ice and subsequently stored at -80°C. To prepare tissue extracts, samples were minced and homogenized in PBS (Phosphate buffered saline). After centrifuging to remove cell debris, the supernatants were collected and the protein concentration was determined using Coomassie-blue protein assay reagent (Pierce Chemical Co., Rockford, IL).

Enzyme-linked immunosorbent assay (ELISA) for MMP-8, MMP-9, TIMP-1 and IL-6

MMP-8 levels in GCFs and levels of MMP-9, TIMP-1 and IL-6 in gingival tissue extracts were measured by using ELISA kits (Amersham Pharmacia Biotech, Little Chalfont, England) according to the manufacturer's instructions. ELISA kits for MMP-8, MMP-9, TIMP-1 and IL-6 had a linear binding curve from 0 to 4 ng/ml, from 0 to 16 ng/ml, from 0 to 400 pg/ml, and from 0 and 50 ng/ml, respectively.

Statistical analysis

All the data were presented as means ± standard deviation (SD) and results were statistically analyzed. Changes in MMP-8 levels from baseline values to each clinical stage (30, 60, 120 days) within each group and inter-group comparison were compared using ANOVA and post-hoc test for multiple comparisons. Changes in

MMP-9, TIMP-1 and IL-6 levels in gingival tissues from baseline values to the final values after 120 days within each group and inter-group comparison were compared using the same method.

III. RESULTS

Patient distribution and Clinical examination

Total of 32 patients with incipient to moderate level of chronic adult periodontitis entered the study. Number of samples at baseline and at the conclusion of the study for clinical and laboratory measurements are as shown (Table 3). Pre-experiment pocket depth of the sites used in the study ranged 4-7mm, with mean pocket depth of 5.4 ± 1.2 mm for SRP + SDD group and 5.5 ± 1.3 mm for SRP + placebo group. There was no statistical difference in pre-experiment pocket depth between the two groups (Table 1). After 120 days, PD, CAL and BOP in both groups enhanced significantly compared to the baseline ($p<0.05$). Especially, statistical significant improvement in PD and CAL was observed in SRP + SDD group (Table 2).

Evaluation of MMP-8 levels in GCF

MMP-8 levels in the GCF from SRP + SDD group and SRP + placebo group were measured every 30 days for 120 days (Figure 1). Initial MMP-8 levels for SRP + SDD group and SRP + placebo group were 407.13 ± 114.45 ng/ml and 378.71 ± 189.39 ng/ml respectively, with no statistical difference between the two groups. MMP-8 levels for SRP + SDD group and SRP + placebo group were: 235.35 ± 134.58 ng/ml and 364.04 ± 219.27 ng/ml at 30 days; 157.50 ± 95.95 ng/ml and 236.60 ± 186.16 ng/ml at 60 days; 102.70 ± 67.64 ng/ml and 208.56 ± 124.54 ng/ml at 90 days;

and 63.77 ± 53.33 ng/ml and 229.13 ± 168.09 ng/ml at 120 days, respectively. MMP-8 levels in both groups gradually decreased as the experiment progressed for 120 days. In SRP + SDD group, the decrease was statistically significant in every 30 days term compared to initial MMP-8 levels, while in SRP + placebo group the difference was not statistically significant. The amount of decrease in MMP-8 levels for SRP + SDD group was statistically significant compared to that for SRP + placebo group, especially apparent at 120 days ($p<0.05$).

Changes in MMP-9 levels in gingival biopsy samples

In SRP + SDD group, there were 7 paired (initial and after 120 days) samples. In SRP + placebo group, there were 11 paired samples. Initial MMP-9 levels for SRP + SDD group and SRP + placebo group were 11.19 ± 6.74 ng/mg protein and 12.97 ± 12.28 ng/mg protein, respectively, with no statistically significant difference. After 120 days, SRP + SDD group showed decreased MMP-9 level to 6.21 ± 3.92 ng/mg protein, while SRP + placebo group showed increased to 19.85 ± 19.15 ng/mg protein, but the differences were not statistically significant (Figure 2). There was no statistically significant difference in MMP-9 levels between SRP + SDD and placebo group ($p <0.05$).

Changes in TIMP-1 levels in gingival biopsy samples

Initial TIMP-1 levels for SRP + SDD and SRP + placebo group were 7.10 ± 6.35 ng/mg, and 5.94 ± 4.72 ng/mg, respectively, with no statistically significant difference.

After 120 days, TIMP-1 levels increased to 15.56 ± 4.33 ng/mg in SRP + SDD group and to 12.78 ± 11.82 ng/mg in SRP + placebo group (Figure 3). In both groups, the increase in TIMP-1 levels from initial value to 120 days value was statistically significant ($p < 0.05$), but there was no significant difference between the two groups.

Changes in IL-6 levels in gingival biopsy samples

Initial IL-6 levels for SRP + SDD and SRP + placebo group were 15.92 ± 7.38 pg/mg, and 16.56 ± 8.67 pg/mg, respectively, with no statistically significant difference. After 120 days, SRP + SDD group showed decreased IL-6 level to 7.38 ± 4.86 pg/mg, and SRP + placebo group also showed decreased slightly to 15.17 ± 9.09 pg/mg (Figure 4). There was no statistically significant difference in IL-6 levels between SRP + SDD and SRP + placebo group.

IV. DISCUSSION

Doxycycline is one of the tetracycline derivatives that have been known for their effectiveness in antibiotic applications. Tetracyclines, due to its effectiveness in suppressing periodontopathic microorganisms[13], have been used widely as adjunctive periodontal therapeutic agents[14]. Compared to other antibiotics, tetracyclines remain in higher concentration in gingival crevicular fluid within the periodontal pockets. Later studies found non-antibiotic effects of tetracyclines which could further extend their clinical applications[15] . Golub et al. suggested that SDD therapy could be used to control and suppress the progression of periodontal disease[4 5]. In addition to these results, Caton et al. reported that SDD therapy combined with scaling and root planing resulted in clinical improvement in chronic adult periodontitis patients[16]. They also showed that this adjunctive SDD therapy did not have antibiotic effect on periodontopathic microorganisms and caused no antibiotic resistance[17]. These findings suggest that adjunctive SDD therapy can be used safely and effectively. This clinical effectiveness of SDD therapy, however, is not well-explained in terms of how SDD reduces periodontal inflammation clinically.

The present study is a double-blinded experiment designed to test the effectiveness of SDD as opposed to placebo in periodontally affected host tissue. This experiment design is used in order to rule out the effect of mechanical periodontal therapy and psychological effect of both the patients and clinicians. After 120 days,

PD, CAL and BOP of the experimental sites improved significantly. These improvements show the effect of mechanical therapy. Changes in PD and CAL, especially, were statistically significant compared to SRP + placebo group, and these changes can be contributed to the effect of 120 day-long adjunctive SDD therapy.

We investigated whether 120 day of SDD therapy in chronic adult periodontitis patients had significant effects on GCF MMP-8 levels, and on gingival tissue MMP-9, TIMP-1 and IL -6 levels. These factors are thought to be the "marker" of periodontal tissue breakdown in chronic adult periodontitis patients[18]. MMPs are the main proteinases related to tissue destruction and remodeling events in periodontal diseases, and recent studies have shown the presence of neutrophil collagenase (MMP-8) and gelatinase (MMP-9) in inflamed human gingiva and GCF of adult periodontitis patients[19]. Studies have shown that MMP-8 and MMP-9 were found in increased level in GCF of chronic adult periodontitis patients[8]. These MMPs are PMN-derived factors, and based on findings that PMN is the origin of destructive enzymes in inflammatory periodontal tissue[20] .

The results showed that the level of MMP-8 within the GCF samples of SRP + SDD group decreased significantly as opposed to those from SRP + placebo group. These findings are consistent with other studies[4 5] which demonstrated that SDD therapy could significantly inhibit collagenase activity in gingival crevicular fluid and in gingival tissue. The differences between the two groups existed in all stage, but became apparent at 120 days of therapy, indicating that the duration of SDD therapy should be long-term in order to be effective.

But, the changes in MMP-9 levels were not statistically significant, even though SRP + placebo group showed increase in MMP-9 level, while SRP + SDD group showed decrease in MMP-9 level.

TIMP-1 is a modulating factor of MMP activity[21 22]. In order to test the effect of TIMP on fibroblast- derived MMPs (MMP-1, 2, 9), the present study evaluated changes in TIMP-1 levels. The results showed that TIMP-1 level increased in both SDD and placebo groups, with no statistically significant difference between the two groups. Increase in TIMP-1 level is probably due to a mechanical intervention that reduces the overall bacterial load in the oral cavity and to a reduction in MMPs which would bind to free TIMP[23 24 25]. It is difficult to conclude the definite effect of SDD therapy on TIMP-1.

IL-6 is a cytokine that is also reported to be closely related to clinical severity of periodontitis. IL-6 is found in high level in periodontal tissue of patients with clinically active periodontitis, and this elevated IL-6 level is shown to be reversed following successful periodontal therapy. In the present study, SRP + SDD group showed greater decrease in IL-6 level compared to SRP + placebo group, although the difference was not statistically significant.

Although it is difficult to make definite conclusion on the positive effect of SDD therapy on MMP-9, TIMP-1, and IL-6, the effect of SDD on MMP-8 level shown in this study can certainly support the clinical value of SDD therapy. Effects on other factors can be evaluated in future studies with larger number of samples, and further studies may be necessary to confirm these results.

In conclusion, adjunctive SDD therapy is an effective mean to control MMP-8 in chronic adult periodontitis patients. Although not statistically significant, SDD therapy also showed inhibitory effects on MMP-9 and IL-6.

V. CONCLUSION

The present study investigated whether sub-antimicrobial dose doxycycline (SDD) therapy for 120d in chronic adult periodontitis patients had significant effects on gingival crevicular fluid MMP-8 levels, and on gingival tissue MMP-9, TIMP-1 and IL-6 levels. A total of 32 patients with incipient to moderate chronic adult periodontitis were randomly assigned to two groups. After scaling and root planning, the SRP + SDD group received SDD, 20 mg bid, whereas the SRP + placebo group received placebo, 20 mg bid.

After 120 days, PD, CAL improved significantly in SRP + SDD group. Initial MMP-8 levels for SRP + SDD group and SRP + placebo group were 407.13 ± 114.45 ng/ml and 378.71 ± 189.39 ng/ml respectively, with no statistical difference between the two groups. MMP-8 levels for SRP + SDD group and SRP + placebo group were: 235.35 ± 134.58 ng/ml and 364.04 ± 219.27 ng/ml at 30 days; 157.50 ± 95.95 ng/ml and 236.60 ± 186.16 ng/ml at 60 days; 102.70 ± 67.64 ng/ml and 208.56 ± 124.54 ng/ml at 90 days; and 63.77 ± 53.33 ng/ml and 229.13 ± 168.09 ng/ml at 120 days, respectively. The amount of decrease in MMP-8 levels for SRP + SDD group was statistically significant compared to that for SRP + placebo group, especially apparent at 120 days ($p<0.05$). TIMP-1 levels in both groups increased from the baseline to 120 days with statistical significance ($p<0.05$), but there was no significant difference

between the two groups. Changes in MMP-9 and IL-6 levels were not statistically significant.

In conclusion, Adjunctive SDD therapy can improve the clinical parameters and this clinical improvement is reflected by controlled level of MMP-8 in chronic adult periodontitis after the therapy.

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Table 1. Clinical evaluations of the experimental sites at the baseline

Group	No.	Age(yr)	Sex	Site	PD(mm)	BOP
	1	33	M	35DL[a]	5	+
	2	39	M	32DL	4	+
	3	43	F	26DL	6	+
	4	34	F	37ML	6	+
	5	46	M	44MB	7	+
	6	45	F	47DB	7	+
	7	64	F	46ML	6	+
SRP + SDD	8	46	M	15DB	4	+
	9	58	M	41MB	4	+
	10	43	F	35DL	6	+
	11	25	F	36DB	5	+
	12	30	M	36DB	7	+
	13	54	M	15ML	4	+
	14	35	M	25ML	6	+
	15	43	F	37DL	4	+
Mean±S.D.		43±10.56			5.4±1.2	
	1	54	M	25DL	6	+
	2	63	M	16DL	6	+
	3	58	F	16ML	6	+
	4	55	F	27DL	6	+
	5	58	F	46ML	4	+
	6	62	M	27DL	4	+
	7	39	M	47MB	6	+
	8	52	M	36ML	4	+
SRP + placebo	9	63	F	26DB	6	+
	10	39	M	25MB	4	+
	11	46	F	25MB	6	+
	12	46	F	17DL	7	+
	13	55	M	17DL	7	+
	14	38	F	35DB	6	+
	15	45	M	15DL	6	+
	16	54	F	17DB	4	+
	17	30	M	16DB	6	+
Mean±S.D.		50±9.80			5.5±1.3	

All values are expressed in mean ± S.D.

M,male, F, female.

[a]Tooth type: MB, mesiobuccal; ML, mesiolingual; DB, distobuccal; DL, distolingual

PD means initial probing depth.

BOP, bleeding on probing; +, presence of BOP; -,absence of BOP

Table 2. Changes of clinical parameters during 120 days

Gourp	Baseline			120 days		
	PD(mm)	CAL(mm)	BOP	PD(mm)	CAL(mm)	BOP
SRP + SDD	5.4 ± 1.2	6.4 ± 0.9	1.0 ± 0.0	3.8 ± 1.5*†	4.2 ± 1.5*†	0.3 ± 0.5*
SRP + placebo	5.5 ± 1.3	6.1 ± 1.1	1.0 ± 0.0	4.4 ± 1.5*	5.5 ± 1.2*	0.5 ± 0.5*

All values are expressed in mean ± S.D.
PD, probing pocket depth (mm).
CAL, clinical attachment level (mm).
BOP, bleeding on probing.
* Statistically significant at $p<0.05$ compared to baseline data.
† Statistically significant at $p<0.05$ compared to SRP + placebo group.

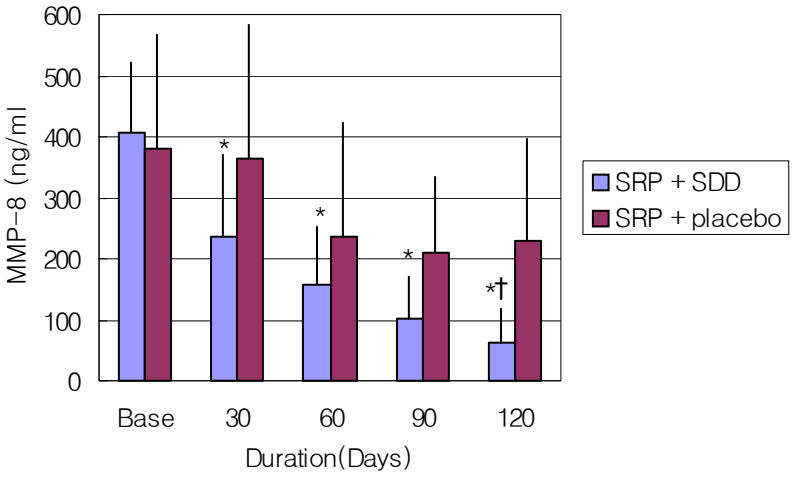

Figure 1. The effect of subantimicrobial dose doxycycline or placebo administration on MMP-8 concentration in gingival crevicular fluid.
* Statistically significant at $p<0.05$ compared to baseline data.
†Statistically significant at $p<0.05$ compared to SRP + placebo group.

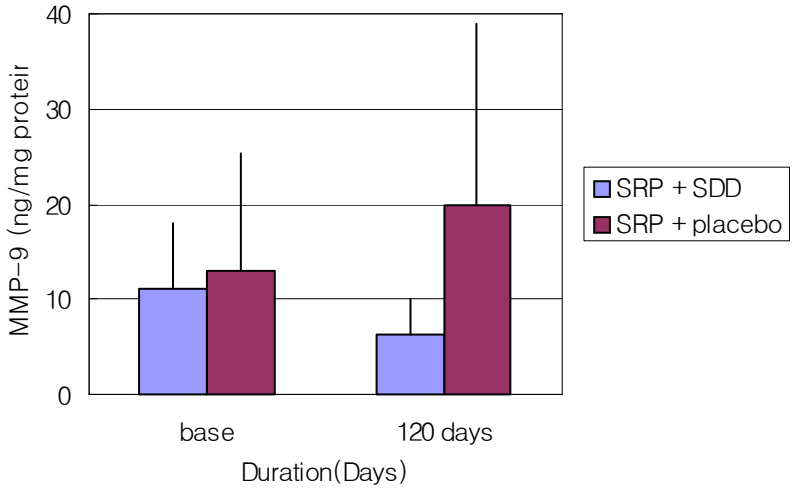

Figure 2. The effect of subantimicrobial dose doxycycline or placebo administration on MMP-9 concentration in gingival biopsies

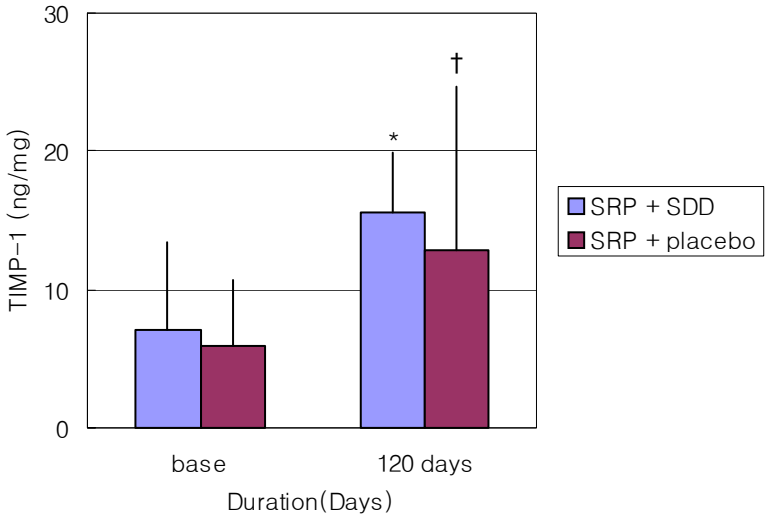

Figure 3. The effect of subantimicrobial dose doxycycline or placebo administration on TIMP-1 concentration in gingival biopsies.
* Statistically significant at $p<0.05$ compared to baseline data.
†Statistically significant at $p<0.05$ compared to baseline data.

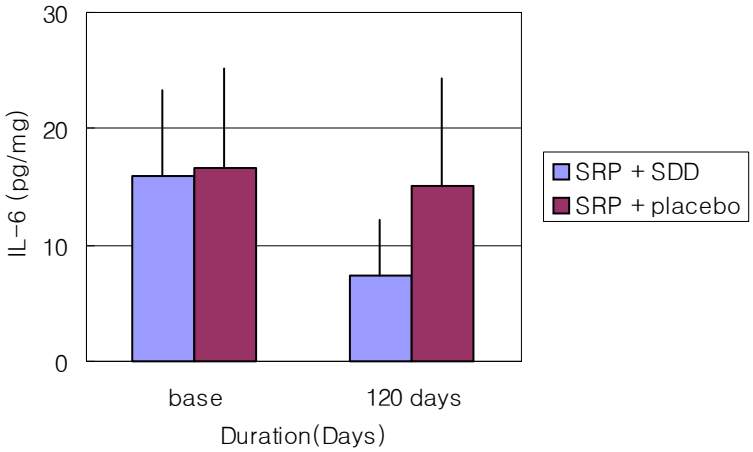

Figure 4. The effect of subantimicrobial dose doxycycline or placebo administration on IL-6 concentration in gingival biopsies.

국문초록

만성 치주염 환자에서 SDD therapy가 치은 열구액 MMP-8, 치은 조직 MMP-9, TIMP-1, IL-6 레벨에 미치는 영향

이 연구는 만성 성인 치주염 환자에서 120일간의 Sub-antimicrobial dose doxycycline therapy (SDD)가 치은열구액의 MMP-8 레벨과 치은조직내의 MMP-9, TIMP-1, IL-6 레벨에 영향을 주는지를 조사하였다.

테트라사이클린은 통상적으로 쓰이는 항생요법보다 낮은 농도로 투여시에도 치은열구액과 치은조직 내의 MMP 활동을 크게 억제할 수 있다. 이러한 SDD 요법은 MMP와 proinflammatory cytokines의 활성을 억제하여 치주질환의 진행을 감소시키는 것으로 나타났다.

총 32명의 incipient to moderate한 성인 만성 치주염 환자(치주낭 깊이 4-7mm)를 대상으로 하였다. 대상 환자들은 랜덤하게 두 그룹으로 나뉘어졌다. 치석 제거와 치근 활택술(SRP) 후, SRP+SDD군은 SDD 20mg bid 투여 하였고 SRP+placebo군은 placebo를 20mg bid 투여하였다. 약물 투여 후 검진시에는 치주낭 깊이 변화(PD), 임상 부착치은 수준 (CAL), 치은 탐침출혈 (BOP), 치은열구

MMP-8 레벨, 치은 조직 MMP-9, TIMP-1, IL-6 레벨 등을 baseline에서 120일 사이에 30일 간격으로 측정하였다.

120일 후 PD와 CAL은 SRP+SDD군에서 유의차 있는 향상을 보였다. SRP+SDD군과 SRP+placebo군의 초기 MMP-8 레벨은 각각 407.13±114.45 ng/ml와 378.71±189.39 ng/ml을 나타내었고 두 군 간의 유의차는 없었다. 약물 투여 후의 SRP+SDD군과 SRP+placebo군의 MMP-8 레벨은 30일 후에 각각 235.35±134.58 ng/ml와 364.04±219.27 ng/ml, 60일 후에 157.50±95.95 ng/ml와 236.60±186.16 ng/ml, 90일 후에 102.70±67.64 ng/ml와 208.56±124.54 ng/ml, 120일 후에 63.77±53.33 ng/ml, 229.13±168.09 ng/ml로 측정되었고 두 군간에 유의차가 있었다. 두 군 모두 TIMP-1 레벨은 baseline에서 120일 간에 유의차 있는 증가를 나타 내었으나 두 군 간에는 유의성 있는 차이를 보이지 않았다. MMP-9과 IL-6 레벨에는 통계적으로 유의성 있는 차이를 보이지 않았다.

보조적인 SDD 요법은 임상 지수의 향상을 가져올 수 있으며 이는 만성 성인 치주염환자에서 치료 후의 MMP-8 레벨의 감소로 나타났다.