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Longitudinal Study of the Subgingival Microbial Change after Tetracycline Topical Application

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=국문초록=

Tetracycline 국소도포가 치은연하 세균분포에 미치는 영향

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최광춘 · 이영희 · 이진용 · 정종평 · 손성희

치주질환의 처치에 있어서 tetracycline의 전신적 투여는 치은연하 세균의 제거 및 감소에 매우 효과가 있는 것으로 알려져 있다. 본 연구는 전신적 투여시의 문제점 보완과 국소적 투여시 기할 수 있는 병소부위의 보다 높은 농도의 유지를 위하여 국소도포용 tetracycline gel을 제조하여 예비실험을 시행하였으며, 본 실험에서는 치주낭 깊이 4~6mm의 중등도 치주염 환자 13명에서 double-blind, split-mouth design으로 상악 또는 하악중 한악은 scaling 및 root planing을 시행하였고, 나머지 악은 그대로 두었으며, 좌측 또는 우측중 한측에는 예비실험결과 가장 효과가 있는 것으로 나타난 국소도포용 3% tetracycline gel을 치주낭내 깊숙히 주2회 2주간 투여하여, ① tetracycline-scaled ② tetracycline-unscaled ③ placebo-scaled ④ placebo-unscaled의 4군에서 0일, 14일, 49일에 임상적, 미생물학적 검사를 시행하여 각기 그 효과를 비교하였다.

또한 치주질환과 관련이 깊은, 그람 음성혐기성 세균, 특히 black-pigmented *Bacteroides*의 분포 변화를 관찰하기 위하여, 시험부위에서 치은연하 치태세균을 채취하여 pre-reduced Ringer액에 혐기성조건으로 보관·운반하여 37℃혐기성 세균 배양기 내에서 혈액배지에 담아 7일간 배양하여 분리한 후, 생화학검사를 통하여 *Bacteroides*균주를 검정하였으며, 이로써 tetracycline투여와 관련된 치주질환의 disease activity를 분석 검토하였다.

이상의 임상적, 미생물학적 검사결과 다음과 같은 결론을 얻었다.

1. 예비실험 결과 국소도포시 가장 효과가 있었던 3% tetracycline gel은 본 실험사용시 치주질환 치료 및 치은연하 세균분포변화에 매우 효과가 있으며, 임상적으로 scaling, root planing과 병행시에 가장 효과가 있는 것으로 나타났다.

2. Bacterial morphotype의 관찰결과 tetracycline투여군에서 coccal form의 증가와 spirochetes의 현저한 감소를 보였으며, non-motile rods와 motile rods의 비율에는 다소증감이 있었으나 의미있는 변화는 없었다.

3. *Streptococcus*균주간의 분포 비율은 tetracycline-scaled군에서 *S. sanguis* I은 14일에 현저히 증가하였고, 49일에는 다소 감소하였으며, *S. mitis*는 증가하는 경향을 보였다.

4. Tetracycline-scaled군에서는 black-pigmented *Bacteroides*의 비율이 현저히 감소하였으며, tetracycline투여만으로도 black-pigmented *Bacteroides*비율은 감소되었다. 그러나 scaling만으로는 black-pigmented *Bacteroides*비율에는 변화를 주지못했다.

5. Black-pigmented *Bacteroides*균주간의 분포를 보면 실험 및 대조군 모두에서 *B. loeschii*가 가장 많이 나타났으며, 시간의 경과에 따라 거의 변화가 없었다. 또한 tetracycline투여군에서는 *B. intermedius*와 *B. gingivalis*가 감소하였고, *B. melaninogenicus*는 증가하였다.

Key Words: Topically applied tetracycline; subgingival microflora

INTRODUCTION

Recent studies indicate that various forms of periodontal disease are associated with relatively specific microorganisms, predominantly Gram-negative anaerobic and capnophilic rods.[13, 20] These findings suggest the possibility of treating periodontal diseases as specific infections.

In the treatment of periodontal disease, the procedures such as subgingival scaling and root planing which aim at the elimination or reduction of the subgingival microbiota lead to improved periodontal conditions.[8] Some investigations indicate that a microbiota normally observed at periodntally diseased sites can be shifted, also by the use of antibiotics such as tetracycline, minocycline, metronidazole, to a flora normally observed at healthy sites.[1] Tetracycline, in particular, has been used for the purpose of antibiotic therapy of periodontal disease. Several reports have described the clinical efficacy of this drug[2,7,11,22] and its effect on the microbial composition of the periodontal pocket. Genco *et al.*[2] reported that the drug significantly enhanced healing in a surgically treated group of severe periodontitis as compared to a group that received the same treatment except tetracycline was replaced by a placebo. Slots *et al.*[22] observed that tetracycline led to an improvement in clinical parameters and a reduction in the microbial flora in some patients that did not respond favorably to conventional therapy.

Kornman and Karl[6] evaluated the effect of long-term low-dose tetracycline therapy on the subgingival microflora in refractory adult periodontitis. The treated patients exhibited a very diverse subgingival microbial flora. Even if no adverse clinical side effects have been reported following systemic tetracycline treatment, the long-term use of antibiotics against dental plaque infections can hardly be justified. Furthermore, discontinuation of active tetracycline therapy involves the risk of the return of a pathological microbial flora and recurrence of disease.[6, 10]

In general, the adverse side effects of antibiotics are minimized if they can be used at the lowest effective doses. Goodson *et al.*[3] and Lindhe *et al*[9] have developed a means of topical application of tetracycline to periodontal pockets by tying hollow fibers filled with tetracycline around periodontally involved teeth. This approach theoretically could utilize locally applied antibiotics at doses far below those used systemically.

The purpose of this study is to compare the clinical and microbiologic effects of (i) scaling and root planing (ii) scaling, root planing, and topical tetracycline therapy, and (iii) topical tetracycline therapy alone, in adults with moderate periodontitis. This study demonstrated the effect of topically applied tetracycline on disease activity of moderate periodontitis.

MATERIALS AND METHODS

Subjects

Thirteen patients, nine males and four females, between the age of 23 and 55 with moderate periodontal disease participated in this experiment. The Ramfjord reference teeth[17] showed a Gingival Inflammatory Index of one or greater and a probing pocket depth of 4 to 6mm. They had not received any antibiotics in the past 6 months, nor did they have any history of allergy,diabetes, blood dyscrasias, or chronic liver or kidney disease. The procedure and risks were explained to all subjects before obtaining their consent to participate in this study.

Clinical Protocol

The clinical trial was a double-blind, split-mouth design. In each patient, two quadrants of the mouth, each randomly selected from the upper and lower jaw, received scaling and root planing. The other half of the mouth was left unscaled. Scaling and root planing of each patient averaged 30 to 45 minutes. No attempt was made to instruct the patients in oral hygiene procedures.

The preliminary project of this study demonstrated that 3% tetracycline gel and 3% tetracycline ointment which were applied topically

in the pocket can lead to an improvement in clinical parameters and a reduction in the subgingival microflora of moderate periodontitis. In this experiment, the 3% tetracycline gel was used because of its convenience in manipulation. In each patient, two quadrants of the mouth selected from the right and left jaw received tetracycline treatment. The tetracycline gel was applied with a syringe tipped with a 23-gauge needle to the periodontal pocket deeply twice a week for two weeks. The other half of the mouth received placebo application.

1. The following clinical measurements were carried out on the Ramfjord teeth; (a) Löe and Silness' Gingival Inflammatory Index,[12] (b) Silness and Löe's Plaque Index,[18] (c) Mühlemann and Son's Sulcus Bleeding Index,[16] and (d) periodontal pocket depth, assessed with a calibrated periodontal probe with markings at each millimeter except for the fourth and sixth millimeters.
2. Gingival crevicular fluid flow was measured with a Harco meter* at the same time each day on the mesial surfaces of the maxillary left central and mandibular right lateral incisors, as follows. After isolation of the teeth with cotton rolls, and drying with a gentle stream of air, a Harco filter paper strip was placed in the pocket to the maximum depth possible for 3 seconds. It was then removed, and another strip was inserted for 60 seconds, whereupon it was removed, and the fluid volume immediately measured with the Harco meter.
3. Attachment level was measured using the cemento-enamel junction as the reference level.
4. Becterial morphotype; Samples were obtained from experimental sites by the use of a curette. Supragingival plaque was removed prior to sampling. The subgingival plaque sample which was suspended in physiologic saline solution was examined in the phase contrast microscope.#

Clinical measurements are obtained at days O(immediately before treatment), 14 and 49. The clinical indices were obtained by the same investigator who, at the time of examination, did not know which quadrants had been scaled and root planed.

Microbiologic Examination

In each patient, the microbial composition was determined for the deepest periodontal pocket in all four quadrants. The microbiologic samples were obtained at the same time as the clinical measurements.

Samples from periondotal pockets were collected by removing supragingival plaque from isolated teeth with sterile cotton balls and three paper points were inserted into the periodontal pocket until resistance was felt. Each was kept in place for 10 seconds and then transferred to 9ml of pre-reduced, anaerobically sterilized Ringer's solution.

Paper point sampling was chosen because this technique, while being relatively reproducible, may not result in a profound disturbance of the subgingival microflora.

The bacterial deposits were dispersed by mixing for 60 seconds in a Vortex mixer at the maximal setting and were then serially diluted in 10-fold steps in Ringer's solution.

For non-selective anaerobic bacterial growth, samples of appropriate dilutions were plated onto blood agar plate of which component is trypticase soy agar@ supplemented with 5% rabbit blood, 0.5μg of hemin per ml, and 0.5μg of menadione (enriched trypticase soy agar) per ml.

For selective anaerobic bacterial growth, samples of appropriate dilutions were plated onto blood agar plate of which component is tryticase soy agar supplemented with 5% rabbit blood, 5.0μg of hemin per ml, and 0.5μg of menadione (enriched trypticase soy agar) per ml and 40μg of Kanamycin per ml.

After 7 days of incubation in an anaerobic

*Periotron 6000, Harco Electronics, Ltd., Winnipeg, Canada

#Olympus BH-2, Olympus Inc. Co., Osaka, Japan

@B. B. L. Microbiology Systems, Cockeysville, M. D., U. S. A.

Table 1. Gingival Inflammatory Index

Patient group	Days after treatment		
	0	14	49
Tetracycline Scaled	2.12±0.55	0.77±0.44*+	0.73±0.73*
Tetracycline Unscaled	1.46±0.48	1.12±0.71*	1.00±0.65*
Placebo Scaled	1.65±0.43	0.86±0.87*	0.92±0.57*
Placebo Unscaled	1.96±0.59	1.81±0.69	1.77±0.42

Mean of Gingival Inflammatory Index ± S.D.

* Statistically significant $p<0.05$, compared to day 0 as determined by T test.

\+ Statistically significant $p<0.05$, compared to placebo groups.

Table 2. Plaque Index

Patient group	Days after treatment		
	0	14	49
Tetracycline Scaled	1.50±0.46	0.77±0.69*	1.00±0.82*
Tetracycline Unscaled	1.81±0.66	1.04±0.80*	1.23±0.78*
Placebo Scaled	1.81±0.69	0.69±0.72	0.77±0.69*
Placebo Unscaled	1.69±0.43	0.88±0.71*	1.38±0.62

Mean of Plaque Index ± S.D.

* Statistically significant $p<0.05$, compared to day 0

chamber* containing 85% N_2, 10% H_2 and 5% CO_2, the total number of colonies and the total number of black-pigmented colonies on the blood agar plates were enumerated.

The proportional recovery was calculated from the medium with the higher number of black-pigmented colonies. Up to 20 black-pigmented colonies selected at random were subcultured from each sample site. Pure culture colonies were grown in the BHI broth for 7 days and 100μl of BHI broth was transferred to PY broth for biochemical test. Using established procedures, each pure culture was identified by Gram stain characteristics, morphology, anaerobiosis, fermentation of glucose, esculin, sucrose, cellobiose, and lactose, production of catalase and indole, hydrolysis of esculin. Metabolic fatty acid end products were determined by gas chromatography (Hitachi model 320).

For isolation and identification of streptocci, samples of appropriate dilutions were plated onto mitis-salivarious agar palte. After 2 days of incubation in an anaerobic chamber, the total number of colonies were counted. From the plate, up to 20 colonies were selected at random and

*Coy Manufacturing Co., Ann Arbor, MI, U.S.A.

were subcultured in the anaerobic chamber for 3 days. Each pure culuture was identified by colonical morphology, Gram staining, hemolytic pattern, catalase test, fermentation of mannitol, sorbitol, raffinose, inulin and lactose, hydrolysis of esculin and L-arginine, acetoin from glucose fermentation.

RESULTS

Clinical Parameters

The clinical results of the experimental groups were compared. The effect of topical tertracycline therapy and scaling on Gingival Inflammatory Index socores is presented in Table 1. Tetracycline therapy resulted in improved gingival health in both the scaled and unscaled groups. However, the greatest reduction in gingivitis score was found when tetracycline therapy was combined with scaling and root planing. The placebo-scaled group also showed reduction in gingivitis score, but the unscaled-placebo group showed no change in ginginval inflammation throughout the study.

The Plaque Index scores are presented in Table 2. The tetracycline groups showed reduction in Plaque Index score. The placebo-scaled and unscaled groups showed a little change at day 49 and 14, respectively.

The remarkable reduction in Sulcus Bleeding Index score was found in tetracycline-scaled group. The data are presented in Table 3.

The pocket depths showed a little reduction in tetracyclin-scaled group at day 49. They showed no significant changes in any group during the experimental period, and are presented in Table 4.

The gingival crevicular fluid data are presented in Table 5. Reduction in gingival crevicular fluid flow was seen in the tetracycline scaled group for up 49 days after treatment. The tetracycline-unscaled and placebo-scaled groups also showed reduction in gingival crevicular fluid flow at day 14, but showed increasing up to baseline level at day 49. No changes were found for placebo-unscaled group.

Prior to treatment, composition of the subgingival microflora of the four experimental groups was compared. Shifts in the subgingival microflora after treatment are shown in Table 6. The tetracycline-scaled group showed the most significant changes in microflora. Treatment resulted in a marked and consistent increase in the proportion of cocci and a corresponding decrease in the proportion of spirochetes. The motile rods also decreased immediately after treatment, and they returned to pretreatment levels at day 49.

The tetracycline-unscaled group also exhibited a rapid reduction in spirochetes and increase in cocci, but the cocci appeared to return to pretre-

Table 3. Sulcus Bleeding Index

Patient group	Days after treatment		
	0	14	49
Tetracycline Scaled	3.12±0.55	1.58±0.53*+	1.65±0.43*
Tetracycline Unscaled	2.46±0.48	1.81±0.78*	1.85±1.05*
Placebo Scaled	2.65±0.43	1.73±0.73	1.69±0.43*
Placebo Unscaled	2.58±0.84	2.35±0.47*	2.19±0.66

Mean of Sulcus Bleeding Index ± S.D.

* Statistically significant $p<0.05$, compared to day 0.

\+ Statistically significant $p<0.05$, compared to placebo groups.

Table 4. Probing pocket depth(mm)

Patient group	Days after treatment		
	0	14	49
Tetracycline Scaled	4.31±0.96	3.62±0.68	3.45±0.47*
Tetracycline Unscaled	3.81±0.68	3.74±0.73	3.56±3.56
Placebo Scaled	4.24±0.96	3.91±0.87	3.58±0.72
Placebo Unscaled	4.44±0.85	3.19±1.01	4.27±0.95

Mean of probing pocket depth ±S.D.

* Statistically significant $p<0.05$, compared to day 0.

Table 5. Gingival crevicular fluid flow measurement (Periotron unit)

Patient group	Days after treatment		
	0	14	49
Tetracycline Scaled	81.39±10.69	53.54± 8.50*+	50.00±12.42*+
Tetracycline Unscaled	62.15±23.58	52.46±18.27*+	62.92±29.45
Placebo Scaled	77.23±42.88	48.77±30.49*+	66.62±33.13
Placebo Unscaled	9.54±19.41	106.38±17.22	98.77±23.07

Mean of gingival crevicular fluid flow±S.D.

* Statistically significant $p<0.05$, compared to day 0.

\+ Statistically significant $p<0.05$, compared to placebo-unscaled group.

ment level earlier in this group than in tetracycline-scaled group. The proportion of motile rods and nonmotile rods changed little during the study.

The placebo-group showed no significant changes in microbial composition as compared to the tetracycline group. Spirochetes were suppressed transiently after treatment in the placebo-scaled group.

Microbiologic Findings

The dominant *Streptococcus* species in four groups were *Streptococcus sanguis* I, *Streptococcus sanguis* II, *Streptococcus mitis* and *Streptococcus intermedius*. The range of organisms encountered was quite extensive. The means of the percentages in relation to the total colony counts of streptococci were presented in Table 7.

The tetracycline-scaled group showed the most significant changes in the proportions of streptococci. The proportion of *S. sanguis* I increased prominantly in this group and that of *S. sanguis* II decreased. *S. mitis* showed moderate increase.

The other three groups showed no major changes in the proportion of streptococci.

The means of the percentages of black-

Table 6. Bacterial morphotype

Patient group	Bacterial group	Days after treatment 0	14	49
Tetracycline	Cocci	31.7±7.2	44.9±3.9*+	38.9±7.5*
Scaled	Nonmotile rods	32.58±8.5	33.5±4.5	33.6±6.8
	Motile rods	19.5±3.8	15.2±3.4*	21.6±2.8
	Spirochetes	16.3±4.9	6.4±3.1*+	5.9±2.9
Tetracycline	Cocci	31.3±5.9	39.0±8.4*+	33.3±9.5
Unscaled	Nonmotile rods	34.2±6.2	31.1±5.8	34.4±6.6
	Motile rods	21.0±4.3	23.0±4.4	23.2±2.4
	Spirochetes	13.5±5.4	6.9±5.6*+	9.1±4.7*
Placebo	Cocci	33.2±4.8	43.9±3.9*+	34.2±8.8
Scaled	Nonmotile rods	32.0±5.5	31.8±5.0	35.0±5.4
	Motile rods	22.2±3.8	18.6±2.3*	21.9±2.6
	Spirochetes	12.6±2.9	6.7±2.9*+	8.9±3.3*
Placebo	Cocci	31.5±6.8	30.5±6.3	30.9±7.7
Unscaled	Nonmotile rods	32.2±6.1	32.2±8.4	34.3±5.5
	Motile rods	24.0±4.9	21.2±5.4	23.5±3.4
	Spirochetes	12.3±3.3	10.1±3.0	11.3±4.4

Mean of the percentages in relation to the total cell counts (mean ± S.D.).

* Statistically significant $p<0.05$, compared to day 0.

\+ Statistically significant $p<0.05$, compared to placebo-unscaled group.

pigmented *Bacteroides* in relation to the total colony counts of anaerobic bacteria were presented in Table 8. The tetracycline groups showed a marked decrease in the proportion of black-pigmented *Bacteroides* at day 49, whereas no changes were found for the placebo group.

In table 9, the proportional mean percentages of black-pigmented *Bacteroides* species among total black-pigmented *Bacteroides* were presented.

All four groups showed the greatest proportion of *B. loeschii* but no major changes between groups. The tetracycline-scaled group exhibited a reduction in *B. intermedius* and *B. gingivalis*. But the proportion of *B. melaninogenicus* increased. The placebo groups demonstrated little change in proportion of black-pigmented *Bacteroides* species.

DISCUSSION

The present study demonstrated that topically applied 3% tetracycline gel improved the gingival health and markedly changed the composition of the subgingival microflora for a prolonged time of period. The greatest effect was seen when tetracycline was given as an adjunct to scaling and root planing, but the use of tetracycline alone also resulted in significant changes of the composition of microflora. Of interest in this study was the finding that tetracycline administration alone induced a significant alteration in the periodontal disease microflora. Loesche *et al.*[15] reported a similar effect of metronidazole to improve the periodontal disease status. In the treatment of periodontal disease, however, antibiotic therapy should be combined with mechanical periodontal debridement. This mode of treatment markedly improves the gingival health and alters the subgingival microflora.

Goodson and Socransky[3] reported that subgingival scaling and root planing produced consipicuous change in the subgingival microflora and the most obvious change in the clinical parameters examined. Furthermore, a comparison between the effects obtained by mechanical

Table 7. Distribution of *Streptococcus* species

Patient group	*Streptococcus* species	Days after treatment		
		0	14	49
Tetracycline Scaled	*S. sanguis* I	30.2 ± 28.4	52.4 ± 37.1*	22.9 ± 35.1
	S. sanguis II	9.7 ± 16.1	7.1 ± 15.2	40 ± 8.3
	S. oralis	4.6 ± 13.4	4.4 ± 9.1	4.1 ± 7.1
	S. mitis	4.9 ± 9.6	9.4 ± 8.4	9.4 ± 2.9
	S. intermedius	27.9 ± 32.5	14.6 ± 20.4	14.2 ± 24.5
	Others	25.2 ± 29.2	22.1 ± 16.2	47.2 ± 32.1
Tetracycline Unscaled	*S. sanguis* I	39.9 ± 38.4	35.4 ± 29.3	18.6 ± 26.7
	S. sanguis II	5.4 ± 8.5	7.5 ± 14.7	7.8 ± 18.3
	S. oralis	0.6 ± 1.7	3.2 ± 5.9	15.5 ± 3.5
	S. mitis	4.9 ± 10.7	6.8 ± 14.6	5.5 ± 11.9
	S. intermedius	10.9 ± 17.3	22.0 ± 34.4	4.7 ± 12.5
	Others	39.2 ± 27.3	27.3 ± 17.8	46.3 ± 32.1
Placebo Scaled	*S. sanguis* I	49.4 ± 37.6	45.9 ± 30.4	16.8 ± 32.7
	S. sangus II	4.4 ± 6.4	10.0 ± 11.0	6.9 ± 13.8
	S. oralis	4.3 ± 9.7	9.2 ± 17.5	11.4 ± 24.4
	S. mitis	4.5 ± 10.8	1.3 ± 2.8	0.3 ± 0.7
	S. intermedius	23.4 ± 30.0	13.4 ± 21.3	4.8 ± 9.9
	Others	14.3 ± 20.3	21.0 ± 28.1	58.9 ± 42.3
Placebo Unscaled	*S. sanguis* I	38.1 ± 32.6	35.3 ± 27.1	39.8 ± 33.3
	S. sanguis II	4.9 ± 9.8	7.9 ± 14.9	3.7 ± 10.1
	S. oralis	5.3 ± 4.2	6.2 ± 9.6	8.8 ± 19.9
	S. mitis	4.8 ± 6.7	4.1 ± 8.7	6.2 ± 11.2
	S. intermedius	30.5 ± 36.4	15.7 ± 28.9	4.3 ± 7.4
	Others	41.3 ± 35.3	31.2 ± 29.3	36.3 ± 31.7

* Mean of the percentages in relation to the total colony counts of streptococci (mean ± S.D.).
Statistically significant $p<0.05$, compared to day 0.

Table 8. Mean of the percentages of black-pigmented *Bacteroides* in relation to the total colony counts of anaerobic bacteria.

Patient group	Days after treatment		
	0	14	49
Tetracycline Scaled	42.5 ± 17.8	34.7 ± 30.7	15.3 ± 12.0*+
Tetracycline Unscaled	40.7 ± 26.1	34.9 ± 27.8	16.7 ± 14.1*+
Placebo Scaled	26.7 ± 24.4	32.3 ± 26.5	21.6 ± 28.7
Placebo Unscaled	41.5 ± 22.2	49.4 ± 24.7	38.2 ± 28.3

* Statistically significant $p < 0.05$, compared to day 0 and both placebo group.

+ Statistically significant $p < 0.05$, compared to placebo groups.

Table 9. Proportions of black-pigmented *Bacteroides* in relation to the total colony counts of BPB (mean ± S.D.)

Patient group	B . P . B	Days after treatment		
		0	14	49
Tetracycline	*B. intermedius*	8.6± 2.3	1.8± 0.3	0.9± 0.4
Scaled	*B. loeschii*	78.6±35.4	80.5±33.2	75.4±31.1
	B. melaninogenicus	9.8± 3.7	13.8± 5.4	18.6± 9.3
	B. gingivalis	2.5± 0.7	1.8± 0.7	0.9± 0.2
	B. denticola	0.4± 0.1	1.2± 0.3	0.9± 0.3
	B. corporis	ND	0.6± 0.1	3.9± 1.4
Tetracycline	*B. intermedius*	4.3± 1.3	2.6± 1.2	1.5± 1.1
Unscaled	*B. loeschii*	84.9±15.2	84.1±23.5	80.9±32.5
	B. melaninogenicus	8.0± 4.3	9.2± 4.2	14.2± 4.8
	B. gingivalis	2.6± 0.7	ND	3.1± 1.7
	B. denticola	ND	ND	ND
	B. corporis	ND	3.9± 1.3	ND
Placebo	*B. intermedius*	6.7± 2.7	3.2± 1.1	4.5± 1.9
Scaled	*B. loeschii*	82.8±16.3	84.7±24.7	77.9±32.8
	B. melaninogenicus	8.7± 4.3	10.0± 4.3	11.9± 4.3
	B. gingivalis	0.7± 0.2	ND	ND
	B. denticola	0.7± 0.3	0.6± 0.2	ND
	B. corporis	ND	1.2± 0.6	5.5± 1.8
Placebo	*B. intermedius*	3.8± 1.2	4.9± 2.8	7.3± 3.2
Unscaled	*B. loeschii*	83.4±19.8	79.5±27.7	80.8±36.3
	B. melaninogenicus	9.5± 3.2	7.3± 4.3	7.3± 3.1
	B. gingivalis	1.2± 0.8	0.8± 0.3	1.4± 0.7
	B. denticola	1.2± 0.7	ND	1.4± 0.4
	B. corporis	0.6± 0.4	7.3± 2.8	1.4± 0.5

and chemical treatment regarding alterations of the subgingival microflora, gingival inflammation and probing depths revealed a close association between the degree of change of the microflora and the degree of improvement of the clinical parameters of periodontitis.

The systemic route of tetracycline is used recently in the thought that it may allow the antibiotic which enters the periodontal pocket with the gingival crevicular fluid to affect microorganisms that will be difficult to reach with topical administration. On the other hand, topical administration through an appropriate delivery system[4,14] has the potential advantage of producing subgingival concentrations which significantly exceed those achievable by systemic administration. The topical tetracycline used in this study is thought to be one of the most effective topical delivery system.

The finding that the Gingival Inflammatory Index and gingival crevicular fluid flow in the tetracycline group did not return to pretreatment levels at a time when the subgingival microflora was markedly suppressed. This may indicate that the observed gingival inflammation was mainly a result of accumulation of subgingival plaque. However, the placebo unscaled group showed a reduction in Plaque Index score. It seemed that it may be resulted from the patients' improved oral hygiene efforts, that might be a placebo effect.

Tanner *et al.*[23] reported that negative correlation existed between *S. sanguis* I and Gram-

negative rods, and *S. mitis* and Gram-negative rods. This agrees with the present study. The tetracycline treatment reduces the proportion of black-pigmentd *Bacteroides* and may result in increase in *S. sanguis* I. Slots and Genco[21] demonstrated the interactions between black-pigmented *Bacteroides* and other oral bacteria. Bacterial antagonism may be a determinant in the oral colonization of black-pigmented *Bacteroides*. *Streptococcus sanguis* elaborates a high-molecular-weight peptide, and *Streptococcus mitis* releases a non-proteinaceous substance which *in vitro* inhibits black-pigmented *Bacteroides*. On the other hand, the black *Bacteroides* pigment, hematin, inhibits *Streptococcus mutans*, and a proteinaceous bacteriocin-like compound from black-pigmented *Bacteroides*, designated melaninocin, inhibits non-melaninocin-producing black-pigmented *Bacteroides, B. oralis, Capnocytophaga, S. mitis., S. salivarius*, and facultative *Actinomyces* species.

The microbiotas recovered from a group of diseases broadly classified as advanced adult periodontitis are also predominated by Gram negative rods including *B. gingivalis, B. intermedius*, other *Bacteroides* species and *F. nucleatum*. Among them, *B. gingivalis*, and *B. intermedius* has been shown to be positively correlated with clinical inflammation in older patients with advanced periodontitis. This organism has been suggested as a possible etiological agent in adult periodontitis.[5,19] In this study, *B. intermedius* occurred in a significantly higher prevalence at the pretreatment day, but decreased after treatment. This result is consistent with other reports.[21,22,23] In general, *B. loeschii* existed predominantly in all experimental groups. Further investigation would be recommended on the prevalence and toxicity of *B. loeschii* and *B. intermedius* in moderate periodontitis.

SUMMARY

Previous studies have developed the technique of topical application of tetracycline(TC) into the periodontal pockets and examined the change of clinical parameters and subgingival microbial morphotypes. The purpose of this study was to longitudinally examine the clinical and microbiological effects of topically applied TC in a double-blind and split-mouth design. Thirteen patients with moderate periodontitis, who were treated with or without TC application and scaling treatment, were examined. TC gel(3%) was used to apply into the selected periodontal pockets twice a week for 2 weeks. During the experiment, clinical parameters and subgingival microbial morphotypes were examined, and for isolation of black-pigmented *Bacteroides*(BPB) and streptococci, an anaerobic sample culturing was done at week 0, 2, and 7. In clinical observation the TC-scaled group exhibited a significant decrease of Gingival Inflammatory Index, Plaque Index, Sulcus Bleeding Index, pocket depth, and gingival crevicular fluid when compared to the TC-unscaled, placebo-scaled, and placebo-unscaled groups. The result of microbial morphotype observation showed a significant increase of coccal form and a decrease of spirochetes in the TC-scaled, TC-unscaled, and placebo-scaled groups. The culture study of streptococci revealed that TC with scaling treatment resulted in a significant increase of *S. sanguis* I at week 2, but its proportion had returned to the base line level. The anaerobic culture study showed that BPB was significantly reduced in the TC-scaled and TC-unscaled groups at week 7. Among BPB species, *B. intermedius* declined significantly with time treatment(week 2 and 7) in the TC-scaled and TC-unscaled groups. These results suggest that the settled pathogenic microflora can be succeeded by nonpathogenic microflora in periodontal pockets after TC treatment.

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