

THE EFFECTS OF TETRACYCLINE-CONTAINING GEL ON GINGIVAL CREVICULAR ENZYME ACTIVITY*

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I. Introduction

The pathogenesis of chronic inflammatory periodontal disease involves destruction of epithelial and connective tissue elements¹⁾. The participation of hydrolytic enzymes as well as the positive correlation between the penetration of inflammatory lesion of periodontal tissue and the quantity of released enzymes have been generally recognized in the pathogenesis of marginal periodontal disease²⁾. These enzymes are released into the extracellular space through labilizing the lysosomal membranes during the phagocytosis the extracellular space and the quantity of enzymes is increased in the inflamed tissue.

Some of the indicators of the host response in gingival crevicular fluid (GCF) appear to be the risk markers for future disease progression as measured by clinical attachment loss. Specifically, indicators of enhanced PMN activity (lysosomal β -glucuronidase, lysosomal collagenase, PMN elastase), PGE₂, and indicators of acute tissue destruction (aspartate

aminotransferase) have been associated with the occurrence of clinical attachment loss³⁻¹⁶⁾.

Depending on the goals of the study, analysis of a specific host mediator in GCF as part of a clinical trial to assess the effectiveness of periodontal therapy is appropriate. The use of a marker that has been associated with occurrence of active disease offers a quantitative measure of the host response, as well as an indication of how effective the therapy may be for reducing the risk of clinical attachment loss during the following 3- to 6- month period.

To date, clinical objective of periodontal therapy has been to halt disease progression. In this regard, clinical trials indicate that scaling and root planing could arrest periodontitis¹⁷⁻²⁶⁾. Both procedures are frequently employed during root instrumentation. However, completed elimination of subgingival deposits using closed procedures is very difficult²⁷⁻³³⁾. Studies utilizing hand instrumentation, and ultrasonic and sonic devices demonstrated that effectiveness of deposits removal dec-

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rease as probing depth increase²⁷⁻³³. When pocketing exceeded 5mm, clinicians often fail to completely debride roots of plaque and calculus, apparently due to decreased accessibility and visibility. Caffesse and coworkers²⁹ reported that roots could be completely cleaned in 83% of the cases when probing depths were 1 to 3mm ; 43% , when the depths were 4- to 6-mm ; and 31% , when the depths were greater than 6mm.

During the 1970s, data derived from *in vitro* experiments suggested that periodontally affected root surfaces contained endotoxin that these substances are cytotoxic to epithelial cells and fibroblast³⁴. Subsequently, it was demonstrated that root planing and ultrasonic devices decreased the quantity of endotoxin to within several nanograms of healthy roots³⁵⁻³⁷. Endotoxin is routinely found on roots of tooth afflicted with periodontitis and, after scaling and root planing, retoxification may occur³⁸. However, excessive cementum removal to provide a root surface free of endotoxin appears to be unnecessary.

Recently, demineralization of root surfaces during periodontal surgery has been performed to enhance regeneration of the lost periodontal attachment and a number of agents have been used for the root demineralization procedure including phosphoric acid³⁹, EDTA⁴⁰, citric acid⁴¹⁻⁴³, and tetracycline⁴⁴⁻⁴⁵.

Tetracycline has many unique advantageous properties other than antimicrobial effect in periodontal disease. It induces root surface demineralization and migration of fibroblast⁴⁶ and periodontal ligament cells⁴⁷ to and on dentin surfaces ; inhibit tissue collagenase production⁴⁸⁻⁵² and *in vitro* bone resorption⁵³ ; and has substantivity^{44,54-56}.

Jeong and coworkers⁵⁷ developed a hydrophilic gels containing tetracycline with or without citric acid and used as root conditioning

agents. The results of clinical trials showed that both gels were able to provide some improvement in gingival health and on the composition of the subgingival microflora, compared to scaling and root planing alone.

The objective of the present study is to assess *in vivo* effects of adjunctive treatment with tetracycline-containing gel on non-surgical periodontal treatment by analyzing clinical parameters and enzyme activity in gingival crevicular fluid.

II. Materials and Methods

Subjects

Twenty-three subjects, with moderate periodontitis 11 males and 12 females, encompassing the ages of 20 and 69 (mean age 42) participated in this study.

The each subject was taken from the following criteria : 1) at least 2 posterior teeth that exhibited probing depth of 4 to 6mm in each jaw quadrant. ; 2) no history of antibiotic therapy and periodontal therapy in the previous 3 months or history of allergy or adverse reaction to tetracycline ; 3) no systemic disease ; and 4) males or non-pregnant, non-lactating females.

Preparation of gels

The gel was prepared using poloxamer (Junsei Chemical Co. Ltd., Seoul, Korea.) in distilled water, was a gelating agent and tetracycline-HCL (Sigma Chemical Co., St. Louis, Missouri.) was added at 50 mg/ml to make 5% tetracycline gel. The pH of the prepared gel was 3.0~3.1⁵⁷.

Clinical Protocol

Ninty-eight posterior teeth from 23 patients were selected for this study. One quadrant of each patient was treated by root planing only (RP group) and another quadrant by root planing and tetracycline-containing gel (RP + TCG group). The gel was applied at the bottom of the pocket using a syringe tipped with a 23-gauge needle. The root surface was actively burnished for 5 minutes using a plastic rubbing instrument specially fabricated for the study to condition it with the gel⁵⁷. After this procedure each site was irrigated with physiologic saline.

Clinical Examination

Clinical parameters were recorded at 0 (immediately before therapy), 4, 12 week. Clinical parameters were examined by the same investigator throughout the study. The plaque index⁶⁰, gingival index⁶¹, and bleeding on probing were recorded. Probing depth and attachment level were assessed with a calibrated periodontal probe to the nearest mm. Attachment level was measured from the cemento-enamel junction to the bottom of the periodontal pocket.

Collection and Processing of GCF samples

Gingival crevicular fluid samples from each site were collected at 0, 1, 2, 4, 12 weeks to determine lactate dehydrogenase (LDH) and beta-glucuronidase (BG) activity.

GCF samples were prepared for enzyme analysis following a standardized collection and processing procedure⁵⁸. The individual crevicular site was gently air-dried and any supragingival plaque was removed. To remove previous fluid, a pre-cut filter paper strip (Ha-

rco Electronics, Winnipeg, Canada) was inserted into the pocket until mild resistance was felt, left in place for 30 seconds and discarded. A new strip was inserted, left in place for 3 minutes and transferred to the Periotron 6000[®] (Harco Electronic, Winnipeg, Canada)⁶²⁻⁶⁴ for GCF-volume determination.

After GCF volume determination, GCF was eluated into 350µl of sterile saline containing 1 percent bovine serum albumin (BSA ; Sigma Chemical Co., St. Louis, Missouri) for 1 hour at 25°C, and the strip was removed and discarded. Samples of the resulting eluate were analysed for lactate dehydrogenase (LDH ; EC 1.1.1.17 ; 40µl) and beta-glucuronidase (BG ; EC 3.2.1.31 ; 50µl). Enzyme activity was recorded as volume activity (concentration ; units/volume GCF).

Calibration of the Periotron 6000[®].

To calibrate the Periotron 6000[®], known volumes of distilled water were evaluated⁵⁸. Using a 1.0µl Microliter syringe (Microcaps, Drummond Scientific), fluid was delivered to pre-cut filter-paper strip in volumes ranging from 0.05 to 1.0µl, at 0.1µl increments. The unit readings on the Periotron 6000[®] were recorded.

Linear regression analysis was used to generate lines representing the relationship of volume to readings on the Periotron 6000[®].

Enzymatic Analysis of Lactate Dehydrogenase

A standard reference assay for detection of LDH activity in serum monitors the oxidation of NADH as the enzyme converts pyruvate to lactate⁶⁵. We analyzed with modified method by Lamster et al⁵⁸. using a commercially-available kit (Lactate dehydrogenase,

Sigma chemical Co., St. Louis, Missouri).

The reactants were mixed and the change in absorbance at 340nm was monitored at 25°C over 3min at 30s intervals on a spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). Disposable half-micro-cuvettes (Polystyrene : 340~800 nm ; light path 10mm ; total capacity 1.5ml ; Semimicro Cuvets, Fisher Scientific) were used. LDH volume activity (International Units (IU)/ml) were calculated.

Enzymatic Analysis of Beta-Glucuronidase

A standard reference assay for BG activity in serum measures the ability of the enzyme to generate phenolphthalein from phenolphthalein glucuronic acid⁶⁶. We determined with modified assay method by Lamster et al⁶⁸.

100µl of 0.075M acetate buffer at pH 4.9, 50µl of 0.03M phenolphthalein glucuronic acid at pH 4.5, 50µl of saline, and 50µl of sample fluid were incubated at 56°C for 2h. The reaction was terminated by adding 350µl of 0.1M 2-amino-2-methyl-1-propanol(AMP) buffer at pH 11 (total volume=600µl). Reaction concentrations were 0.03M buffer and 6 mM substrate, and final concentrations of AMP buffer were 58mM.

The absorbance at 550nm was measured against a reagent blank, and compared to a phenolphthalein standard curve to obtain concentration results which were used to calculate the BG volume activity (Units/ml per h ; one unit=1µg of phenolphthalein released at 56°C).

All absorbance readings were made on Spectrophotometer using disposable half-micro-cuvettes. Concentrations of phenolphthalein ranging from 2.0 to 8.0µg/ml were plotted against absorbance at 550nm and linear regression was used to determine the line of best fit. All chemicals and reagents were purchased

from Sigma Chemical Co., St. Louis, Missouri.

Statistical Analysis

All data are reported as mean \pm standard deviation. For all clinical and enzymatic parameters, differences from the baseline value (week 0) to proceeding week, and two time points were evaluated by the two-tailed *t* test. Differences are presented as $p < 0.05$ and $p < 0.01$.

III. Results

Clinical Parameters

The mean values for plaque index (PI score) were presented in Table 1 and Figure 1. In both groups, significant reduction in PI scores were observed at the 4 week and 12 week ($p < 0.01$). There were no significant differences in plaque index scores between two groups. The gingival index scores were presented in Table 2 and Figure 2. The GI scores were significantly reduced at the 4 week and maintained to 12 week ($p < 0.01$). In RP group, the reductions were greater but there were no statistically significant. Table 3 and Figure 3 presented the bleeding on probing as percentage value. In both groups, bleeding sites reduced significantly at 4 and 12 week ($p < 0.01$). RP+ TCG group showed more bleeding sites, but there were no statistically significant differences between the groups. The probing depth were shown in Table 4 and Figure 4. Both groups exhibited significant reductions in probing depth at 3 week and maintained to 12 week ($p < 0.01$). The reductions were greater in RP group but there were no statistically significant differences compared to RP+ TCG group. There were continuous significant improvements in attach-

Table 1. Plaque Index (mean \pm SD)

Week	RP	RP+TCG
0	1.33 \pm 0.63	1.37 \pm 0.59
4	0.69 \pm 0.78* ^s	0.44 \pm 0.46* ^s
12	0.50 \pm 0.58* ^s	0.69 \pm 0.67* ^s

*Significantly different from time 0 ($p < 0.01$)

^sSignificantly different from previous week ($p < 0.01$)

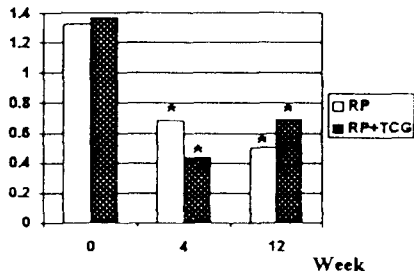


Fig.1 Plaque Index

*significantly different from time 0 ($p < 0.01$)

Table 3. Bleeding on Probing (mean \pm SD)

Week	RP	RP+TCG
0	0.94 \pm 0.22	9.42 \pm 0.23
4	0.39 \pm 0.40* ^s	0.57 \pm 0.44* ^s
12	0.46 \pm 0.43* ^s	0.60 \pm 0.38* ^s

*Significantly different from time 0 ($p < 0.01$)

^sSignificantly different from previous week ($p < 0.01$)

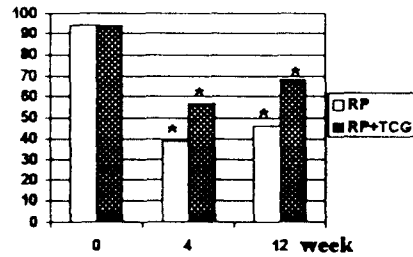


Fig.3 Bleeding on Probing

*significantly different from time 0 ($p < 0.01$)

Table 2. Gingival Index (mean \pm SD)

Week	RP	RP+TCG
0	2.50 \pm 0.69	2.45 \pm 0.61
4	0.60 \pm 0.85* ^s	0.77 \pm 0.75* ^s
12	0.56 \pm 0.61* ^s	0.64 \pm 0.68* ^s

*Significantly different from time 0 ($p < 0.01$)

^sSignificantly different from previous week ($p < 0.01$)

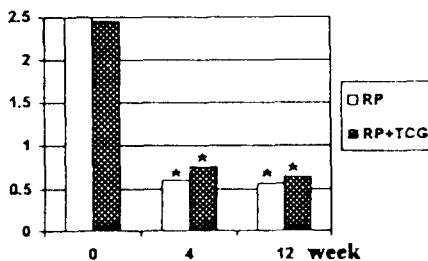


Fig.2 Gingival Index

*significantly different from time 0 ($p < 0.01$)

Table 4. Probing depth (mean \pm SD ; in mm)

Week	RP	RP+TCG
0	5.09 \pm 0.70	5.08 \pm 0.74
4	3.44 \pm 0.69* ^s	3.82 \pm 1.78* ^s
12	2.88 \pm 0.62* ^s	3.24 \pm 1.12* ^s

*Significantly different from time 0 ($p < 0.01$)

^sSignificantly different from previous week ($p < 0.01$)

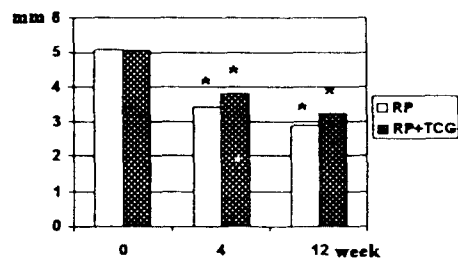


Fig.4 Probing depth

*significantly different from time 0 ($p < 0.01$)

ment level throughout the study compared to baseline in both groups (Table 5 and Figure

5). Although significant differences were not detected, RP group had more attachment

Table 5. Attachment level (mean \pm SD ; in mm)

Week	RP	RP+TCG
0	5.35 \pm 1.07	5.43 \pm 0.93
4	4.28 \pm 0.87* [§]	4.56 \pm 1.876* [§]
12	3.37 \pm 0.74* [§]	3.78 \pm 1.04* [§]

*Significantly different from time 0 (p<0.01)

[§]Significantly different from previous week (p<0.01)

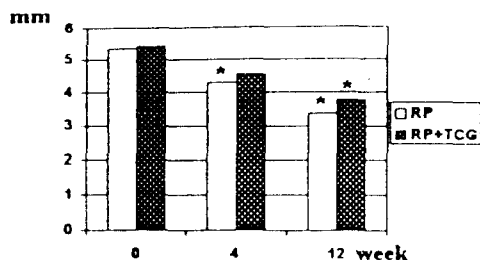


Fig.5 Attachment level

*significantly different from time 0 (p<0.01)

Table 7. LDH Activity (mea \pm SD ; in μ l/ml)

Week	RP	RP+TCG
0	218.8 \pm 141.5	213.8 \pm 120.5
1	192.4 \pm 114.4	185.1 \pm 106.2
2	179.0 \pm 143.0	156.4 \pm 121.0
4	130.6 \pm 62.4* [§]	122.6 \pm 62.8* [§]
12	121.6 \pm 91.9* [§]	95.2 \pm 38.6* [§]

*Significantly different from time 0 (p<0.05)

[§]Significantly different from previous week (p<0.05)

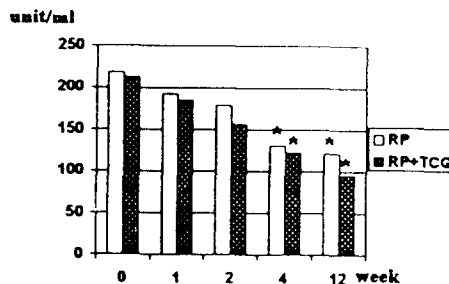


Fig.7 Lactate dehydrogenase activity

*significantly different from time 0 (p<0.05)

Table 6. GCF Volume (mean \pm SD ; in μ l)

Week	RP	RP+TCG
0	0.36 \pm 0.27	0.49 \pm 0.29
1	0.36 \pm 0.21	0.36 \pm 0.22
2	0.32 \pm 0.25	0.28 \pm 0.19* [§]
4	0.23 \pm 0.16	0.26 \pm 0.27* [§]
12	0.32 \pm 0.29	0.34 \pm 0.28

*Significantly different from time 0 (p<0.01)

[§]Significantly different from previous week (p<0.01)

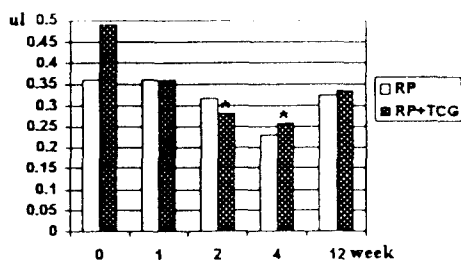


Fig.6 GCF volume

*significantly different from time 0 (p<0.01)

Table 8. BG Activity (mean \pm SD ; in μ l/ml)

Week	RP	RP+TCG
0	10.2 \pm 10.7	14.3 \pm 17.4
1	5.4 \pm 4.6	4.5 \pm 8.0* [§]
2	2.5 \pm 2.4* [§]	2.1 \pm 1.9* [§]
4	1.9 \pm 2.2	3.1 \pm 4.2* [§]
12	2.8 \pm 1.8	4.4 \pm 3.0* [§]

*Significantly different from time 0 (p<0.05)

[§]Significantly different from previous week (p<0.05)

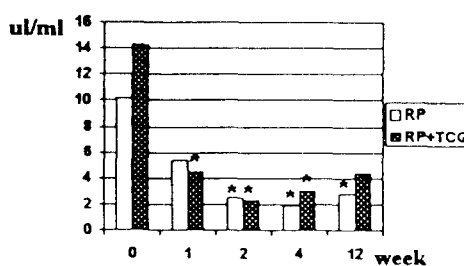


Fig.8 BG Activity

*significantly different from time 0 (p<0.05)

gains. Mean values of GCF volume were presented in Table 6 and Figure 6. In both groups, the values were reduced to 4 week and slightly increased at 12 week. At 2 and 4 week, significance was detected in RP+TCG group.

Enzyme Activity of LDH

LDH activity was reduced throughout the study and significant difference was detected at 4 and 12 week (Table 7 and Figure 7). Although more pronounced reduction was observed in RP+TCG group, there were no statistical significance compared to RP group.

Enzyme Activity of BG

The mean value of BG volume activity was significantly reduced throughout the study (Table 8 and Figure 8). Comparing to the RP group, there were no significant differences but the reductions were greatly pronounced in the RP+TCG group.

IV. Discussion

The purpose of this study was to evaluate the effect of tetracycline-containing gel used as a root conditioning agent following non-surgical therapy on clinical parameters and gingival crevicular enzyme activity. The results indicated that tetracycline-containing gel had no additional improvement in clinical gingival health. Although statistical significances were not found, more reductions of lactate dehydrogenase activity and beta-glucuronidase activity in GCF of root planing plus tetracycline-containing gel treated group compared to scaling and root planing therapy alone were detected.

The reason of this results might be explained by the three ways.

First, the effect of root planing significantly pronounced and the influences of tetracycline-containing gel could be hindered. The scores of clinical parameters were significantly reduced and maintained or continuously reduced throughout the study. The present results in agreement with those of several previous reports⁶⁷⁻⁷¹, showed that comprehensive scaling and root planing is capable of arresting periodontal destruction, and maintain periodontal health.

In our study, root planing was performed under local anesthesia, and one quadrant per one visit. Also, the subgingival scaling and root planing was carried out by an experienced periodontist. Thus, average of 10min was spent per tooth, and several lesions required more time until the instrumentation had resulted in a clinically clean, smooth and hard surface when it was examined by explorer. This extended scaling period seemed necessary because the debridement took place without concomitant periodontal surgery. Also repeated oral hygiene instruction and professional plaque control at 4, 12 week could influences on this results. Many investigators have suggested that there were no advantage to the use of antimicrobial agents in treating patients who are capable of effective oral hygiene, if they have undergone meticulous subgingival debridement⁷²⁻⁷⁴.

Secondly, confident gel application into deep pocket might not be appropriate. Because of viscous characteristics of gel, it was not possible that gel was inserted into all the periodontal pocket area. In fact, gel insertion into pocket by needle had some difficulties. Jeong et al⁵⁷, explained that conventional use of aqueous solution of citric acid of tetracycline would have caused several problems in their study, such as difficulty of long-term storage, uncertainties of proper application of agents

into the bottom of the pocket and to root surfaces, and confinement at one site to prevent spillover. Further evaluation are required in characteristics of gel and the method of gel application.

Third, it seems likely that active stroke with specially designed instrument for the purpose of root conditioning effects on periodontal healing or involves soft tissue damage. Infact, Factly, some bleeding were observed during application times. Bleeding on probing, for example, although significant differences were not observed between two groups, in RP+TCG group showed more bleeding sites. Also less improvement of pocket depth reductions and attachment gains in RP+TCG group could be explained by this reason. Compared to previous study⁷⁵⁾, it isn't like that gel itself induces soft tissue damage.

The results of our analysis of the calibration of the Periotron 6000[®] agree in large part with those of previous study^{58,62)}, who demonstrated that each unit reading on the Periotron 6000[®] corresponded to approximately 0.005 μ l when volumes from 0.15 μ l to approximately 0.6 μ l were analysed.

GCF flow rate is dependent on vascular permeability and is one of the most extensively studied indicators of inflammatory changes in gingival tissues. Several studies have demonstrated a correlation between GCF flow rate and clinical indices of gingival inflammation⁷⁶⁻⁷⁹⁾ which has been confirmed by most studies that have evaluated the association of GCF flow with histologic findings of inflammation^{77,79)}. Our study demonstrated that GCF volumes significantly reduced in RP+TCG group at 2 and 4 week.

Lactate dehydrogenase is cytoplasmic enzyme and marker of cell death or necrosis. Several studies^{6,59,80)} have indicated a positive correlation between enzyme levels and inflam-

mation, however definitive demonstrations of association with active destruction are limited. Our results indicated that LDH enzyme activity in GCF continuously reduced throughout the study, and significant differeces were detected at 4 and 12 week.

Beta-glucuronidase is designated in the literature as tracer of lysosomal organelles. In the catabolism of acid mucopolysaccharrides, beta-glucuronidase is probably responsible for the final degradation of the oligosaccharrides produced initially by the action of hyaluronidase.

In longitudinal study, the activity of BG was significantly elevated during monitoring periods that coincided with attachment loss of 2mm or more. Evaluation of this lysosomal enzyme activity in GCF could a more sensitive indicator of tissue status than traditional clinical parameters^{3,4,6-8,16)}. In this study represented that BG activity significances were not found, more reductions of LDH and BG activities were detected in RP+TCG group.

Considering this limited reduction of GCF volume, LDH and BG activities in RP+TCG group, tetracycline-containing gel seems likely to have the effect on GCF envioronment.

Tetracyclines and their chemically modified analogues have been shown to inhibit the activity of the matrix metalloproteinase (MMP), collagenase. But the mechanisms by which tetracyclines affect and, possibly, decrease bone resorption are not yet clearly understood. Rifkin et al⁸¹⁾. demonstrated a number of possibilities, i.e., 1) directly inhibit the activity of extracellular collagenase and other MMPs such as gelatinase. ; 2) prevent the activation of its proenzyme by scavenging reactive oxygen species generated by other cell types. (e.g. PMNs, osteoclasts) ; 3) inhibit the secretion of other collagenolytic enzymes. (i.e. lysosomal cathepsins) ; and 4) directly affect other

aspects of osteoclast structure and function.

The results of present study suggested that pronounced improvement in clinical parameters or GCF enzyme activities were not found in RP+TCG group when compared to RP group. Only limited effect on GCF enzyme activities and decreased GCF volume were detected in RP+TCG than RP group. Further investigations are suggested to verify the long-term effects of gel on the periodontal tissue, application method and frequency and adverse effects.

V. Conclusions

In this investigation, the clinical effects and enzyme activities in gingival crevicular fluid of root planing with or without root conditioning of tetracycline-containing gel were evaluated.

The following conclusions were as follows :

1. In root planing group (RP group), and root planing followed by root conditioning with tetracycline-containing gel treated group (RP+TCG group), plaque index, gingival index, bleeding on probing and pocket depth were significantly decreased and there was a significant gain of attachment level. But there were no significant differences between two groups.
2. The RP+TCG group represented significant reduction in GCF volume at 2 and 4 week than RP group.
3. LDH and BG activity in GCF were significantly reduced in both groups. But there were no statistical significances between two groups. However slightly more reductions were founded in RP+TCG group.

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치근활택술 및 테트라사이클린 젤의 부가적 사용시 치은 열구액내 효소활성도에 관한 연구

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본 연구의 목적은 비외과적 치주치료시에 부가적으로 사용하기 위해 개발한 테트라사이클린 젤의 효과를 임상지수, 치은 열구액양 및 치은열구액내 효소활성도 측정을 통해 평가함에 있다.

치주낭 깊이가 4mm 이상 6mm 이하인 구치가 편약당 적어도 2개 이상 존재하는 중등도 치주염에 이환된 환자 23명을 선택하여, 한 편약에서는 치근활택술 만을 시행하였고, 다른 한 편약에는 치근활택술 후 테트라사이클린 젤을 치은연하로 주입한 뒤 치근면 처리하였다. 사용된 임상검사 지수는 치태지수, 치은지수, 치주낭 깊이, 치은부착 상실도 및 탐침시 출혈로, 각각 치료시작 전과 치료 후 4, 12주에 검사하였으며, 치은열구액내 효소활성도 검사를 위해 치은열구액을 치료시작 전과 치료 후 1, 2, 4, 12주에 각각 채취하고 치은열구액 양을 조사한 뒤 Lactate dehydrogenase(LDH)와 Beta-glucuronidase(BG)의 활성도를 측정하였고, 효소활성도는 LDH는 340nm에서, BG는 550nm에서 각각 흡광도를 조사 분석하여 다음과 같은 결과를 얻었다.

1. 임상지수를 측정 비교한 결과 치근활택술 만으로 치료한 군과 치근활택술 후 테트라사이클린 젤을 병용한 군 모두에서 임상적으로 치은건강의 현저한 향상을 관찰할 수 있었으나, 통계상 두 군간의 유의성있는 차이는 발견되지 않았다.
2. 치은열구액 양은 치근활택술 후 테트라사이클린 젤을 병용한 군이 치근활택술만 시행한 군보다 치료 후 2주와 4주에 유의성있게 감소하였다.
3. LDH와 BG 효소 활성도는 두 군 모두 현저히 감소하였고, 비록 통계학적인 유의성은 관찰할 수 없었으나 치근활택술 후 테트라사이클린 젤을 병용한 군에서 감소량이 더 크게 나타났다.

주요어 : 테트라사이클린 젤, 치은열구액내 효소활성도, Lactate dehydrogenase, Beta-glucuronidase