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Effect of Scaling and Root Planning with Topical Doxycycline *versus* Scaling and Root Planning only on the Subgingival Microbiota of Chronic Periodontitis Patients

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Abstract. The objective of the present investigation is to compare the effect of topical application of Doxycycline 10% (Atridox^{TM}) following scaling and root planning, thus, versus scaling and root planning alone on the subgingival plaque composition of patients with generalized severe chronic periodontitis. Using split mouth design, subgingival plaque samples were taken at the baseline and 2 months after treatment. Microbiological analysis was carried out using checkerboard DNA probe technology. Plaque samples were screened for the presence of *Actinomyces naeslundii, Tannerella forsythia, Campylobacter conscisus, Campylobacter curva, Campylobacter rectus, Capnocytophaga sputigena, Eikenella corrodens, Fusobacterium nucleatum ss vincentii, Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Streptococcus oralis, Treponema denticola, and Aggregatibacter actinomycetemcomitans serotype b.* After two months post-treatment, there was a significant decrease in total bacterial counts in both groups. Pathogens such as *Tannerella forsythia, Porphyromonas gingivalis and Treponema denticola* were reduced significantly when Atridox^{TM} was used in addition to scaling and root planning, while *Actinomyces naeslundii* and *Streptococcus oralis* levels were increased. The topical application of Doxycycline 10% as an adjunct to scaling and root planning is beneficial to the non-surgical management of patients with generalized severe chronic periodontitis.

Keywords: Chronic Periodontitis, Doxycycline hyclate, Atridox^{TM}, Scaling and root planning.

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Introduction

Subgingival plaque composes mainly of gram-negative anaerobic microorganisms. It is believed that the composition of the subgingival plaque coupled to the inability of the host to overcome the bacterial challenge will lead to periodontal destruction[1]. Periodontal treatment consists of eliminating plaque *via* mechanical methods such as scaling and root planning, or chemical methods such as antiseptic rinses, systemic antibiotic therapy, or a combination of the two. The most frequently used family of antibiotics has been the tetracyclines, which combines large spectrum activity on oral microorganisms as well as inhibiting anticollagenolytic properties[2]. Systemic antibiotic therapy presents inconveniences such as poor patient compliance, emergence of allergies and development of bacterial resistance to antimicrobial drugs. In the 1980's, the delivery of antimicrobial agents directly into periodontal pockets, consequently avoiding the systemic route, began to be used in the periodontal community[3].

The application of antimicrobials subgingivally, historically started with subgingival irrigation. Periodontitis have irrigated subgingival pockets with dilutions of Sodium hypochlorite, Povidine-iodine, Chlorhexidine gluconate, and other antimicrobials or antiseptics[4]. This process involved local delivery, but none controlled-release. Controlled-release local delivery systems, for which the antimicrobial agent was available at therapeutic levels for several days, have been evaluated in several forms and using different antimicrobials. The first one to appear on the market was Actisite™ (Tetracycline HCL)[5], followed by Periochip™ (Chlorhexidine gluconate)[6], Atridox™ (Doxycycline Hyclate)[7,8], and Arestin™ (minocycline)[9]. These systems appear to hold some promises in non-surgical periodontal therapy, since they have the ability to reduce the bacterial load present subgingivally. This also set the stage for a different microbial colonization that would be more compatible with periodontal health.

Atridox™ presents in the form of a biodegradable, flowing gel that solidifies when in contact with humidity. Each reconstituted syringe contained 42.5 mg of doxycycline hyclate. In two well-controlled, multicenter, parallel-design, nine month clinical trials studies enrolling 831 patients with chronic periodontitis, the investigators compared the efficacy of Atridox™ against scaling and root planing (SRP), oral hygiene

and placebo[7,8]. At 6 months Atridox™ was as effective as SRP in reducing pocket depth. Also 6 months following an Atridox™ application, 70% of anaerobic bacteria had not reestablished themselves[10]. Up to date, the outcome of Atridox™ application in deep periodontal pockets following SRP in severe chronic periodontitis patients has not yet been investigated.

The purpose of the present investigation was to compare the subgingival microbiota after SRP, followed by topical application of a Doxycycline hyclate 10% gel (Atridox™), with SRP alone, in patients with generalized severe chronic periodontitis.

Materials and Methods

A total of 20 patients (10 males, 10 females, aged 40-60 years) with severe chronic periodontitis (N = 80 teeth) were selected for this study. Using a split mouth design, one side of the mouth received SRP under local anesthesia followed by application of topical doxycycline hyclate 10% gel (Atridox™) [tested teeth (N = 40)] while the contralateral side, which served as control was treated with SRP alone [control teeth (N = 40)]. After isolating the teeth with cotton rolls, subgingival plaque samples were taken with sterile Gracey curette at the baseline and two months after the treatment in order to carry out microbiological analysis. The subgingival plaque samples were taken from the mesiobuccal aspect of the test and control teeth in each subject. Counts of 14 subgingival species were determined in each plaque sample using the checkerboard DNA-DNA probe technology[10,11]. Plaque samples were screened for the presence of: *Actinomyces naeslundii (A. naeslundii)* strain 43146; *Tannerella forsythia* (*T. forsythia*) [*ex-Bacteroides forsythus*] strain 338; *Campylobacter conscisus* (*C. conscisus*) strain 484; *Campylobacter curva* (*C. curva*) strain 9584; *Campylobacter rectus* (*C. rectus*) stain 371; *Capnocytophaga sputigena* (*C. sputigena*) stain 33562; *Eikenella corrodens* (*E. corrodens*) strain 23834; *Fusobacterium nucleatum* (*F. nucleatum*) *ss vincentii* strain 364; *Porphyromonas gingivalis* (*P. ginigivalis*) strain 381; *Prevotella intermedia* (*P. intermedia*) strain 25611; *Prevotella nigrescens* (*P. nigrescens*) strain 33563; *Streptococcus oralis* (*S. oralis*) strain SSII, *Treponema denticola* (*T. denticola*); *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) serotype b strain Y4.

Positive signals were identified by chemiluminescence as described by Haffajee *et al.*[12]. Presence or absence of a particular microorganism was determined by the presence or absence of a black spot (positive signal) on the radiographic film. A semi quantitative analysis was carried out by comparing the intensity of the signals to 2 sets of controls (known amounts of bacterial DNA) present on the film. These controls represented bacterial counts of 10^5 and 10^6, respectively. Signals were evaluated visually by comparison with the standard for the test species. They were recorded as: 0 = not detected; 1 ≤ 10^5 cells; 2 = 10^5 cells; 3 = 10^5 to 10^6 cells; 4 = 10^6 cells; 5 ≥ 10^6 cells

Results

Results in the graphs are expressed as mean ± Standard Deviation (SD). For statistical analysis, Wilcoxon Signed Rank test was conducted to evaluate significant changes in the bacterial count between the two groups using the differences in counts from pre- to post-treatment. To evaluate the changes in the total bacterial counts at the end of the study, the Mann-Whitney U test was used. The difference between groups was considered significant for a p value < 0.05.

There was a significant decrease in total bacterial counts post-treatment in both groups (Tables 1 and 2). Scaling and root planning with or without Atridox™ proved to be efficient in reducing the bacterial load.

Table 1. Bacterial count before and after scaling and root planning alone in the control group (Mean ± Standard Deviation).

PATHOGEN	Pre-treatment	Post-treatment	p Value
Actinomyces naeslundii	1.27 ± 0.38	2.73 ± 0.56	< 0.001
Tannerella forsythia	3.46 ± 0.90	1.40 ± 0.52	< 0.001
Campylobacter conscisus	3.00 ± 0.66	1.00 ± 0.25	< 0.005
Campylobacter rectus	3.00 ± 0.72	0.93 ± 0.19	< 0.005
Capnocytophaga sputigena	0.00 ± 0.00	0.00 ± 0.00	0.000
Eikenella corrodens	3.60 ± 0.82	1.20 ± 0.46	< 0.005
Fusobacterium nucleatum ss vincentii	2.80 ± 0.77	1.21 ± 0.37	< 0.001
Porphyromonas gingivalis	3.93 ± 0.57	1.26 ± 0.30	< 0.001
Prevotella intermedia	3.46 ± 0.59	1.06 ± 0.30	< 0.001
Prevotella nigrescens	2.86 ± 0.78	1.06 ± 0.38	< 0.001
Streptococcus oralis	2.26 ± 0.78	3.33 ± 0.64	< 0.005
Treponema denticola	3.73 ± 0.64	1.20 ± 0.37	< 0.001
Campylobacter curva	1.33 ± 0.41	0.33 ± 0.11	< 0.005
Aggregatibacter actinomycetemcomitans serotype b	0.66 ± 0.31	0.20 ± 0.11	< 0.01

Table 2. Bacterial count before and after scaling and root planning with Atridox™ in the test group (Mean ± Standard Deviation).

PATHOGEN	Pre-treatment	Post-treatment	p Value
Actinomyces naeslundii	1.26 ± 0.42	3.80 ± 0.54	< 0.005
Tannerella forsythia	3.73 ± 0.76	0.60 ± 0.23	< 0.005
Campylobacter conscisus	2.86 ± 0.61	1.13 ± 0.51	< 0.005
Campylobacter rectus	3.00 ± 0.52	0.86 ± 0.24	< 0.005
Capnocytophaga sputigena	0.00 ± 0.00	0.00 ± 0.00	0.000
Eikenella corrodens	3.63 ± 0.40	1.21 ± 0.39	< 0.005
Fusobacterium nucleatum ss vincentii	2.93 ± 0.56	1.13 ± 0.34	< 0.001
Porphyromonas gingivalis	4.06 ± 0.39	0.40 ± 0.13	< 0.001
Prevotella intermedia	3.47 ± 0.58	1.05 ± 0.25	< 0.005
Prevotella nigrescens	2.87 ± 0.77	1.13 ± 0.39	< 0.005
Streptococcus oralis	2.26 ± 0.75	4.60 ± 0.98	< 0.001
Treponema denticola	3.30 ± 0.44	0.53 ± 0.18	< 0.005
Campylobacter curva	1.34 ± 0.40	0.33 ± 0.12	< 0.01
Aggregatibacter actinomycetemcomitans serotype b	0.69 ± 0.30	0.20 ± 0.11	< 0.01

When comparing the results of treatment, scaling and root planning versus SRP plus Atridox™, there was statistical difference. These differences were statistically significant ($p < 0.05$) (Fig. 1). Microorganisms such as *T. forsythia, P. gingivalis* and *T. denticola* were significantly reduced while beneficial species such as *A. naeslundii* and *S. oralis* were increased (Fig. 2). The adjunction of Atridox™ to scaling and root planning seemed to have a greater effect in reducing periodontal pathogens from the subgingival microbiota. These microorganisms being part of the "red complex" described by Socransky *et al*.[13]; a significant reduction of these periodontal pathogens is likely to lead to a shift in the subgingival microbial composition that is more conductive to periodontal health. Likewise, the increase in beneficial species such as *Streptococci* and *Actinomyces* is more adapted to clinical health.

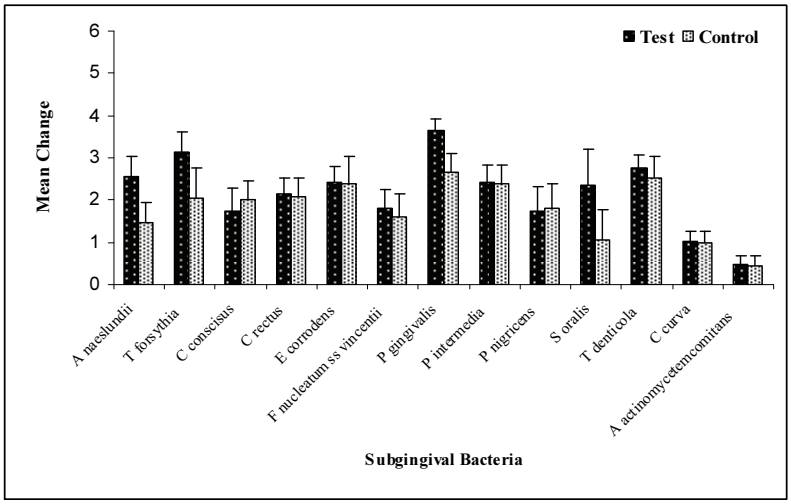

Fig. 1. Mean change in levels of potential pathogens (between test and control groups).

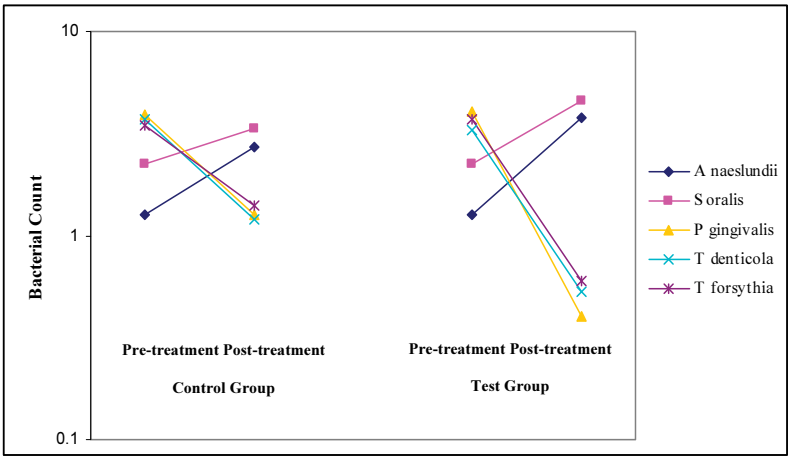

Fig. 2. Comparative bacterial counts in the test and control groups before and after treatment.

Discussion

Periodontal disease progression requires the simultaneous presence of a high number of pathogens, low numbers of beneficial species, a conductive local environment, and a susceptible host[14]. Therefore, successful therapy was linked to the alteration of one or more of the above mentioned factors. Elimination or suppression of the periodontal pathogens;

shifting the subgingival microbiota to one that includes high numbers of beneficial species (such as *Actinomyces, Streptococci* and *Veillonella*); changing the local environment to one that impairs disease progression, and finally altering the host's immune response to one that is less prone to infection, are all acceptable treatment strategies.

The goal of this study was to act directly on the first two parameters (suppression of the periodontal pathogens and shifting of the subgingival flora) *via* SRP can remove up to 90% of the subgingival microorganisms in a subgingival pocket[15]. This alone can elicit long standing changes in the bacterial plaque. In addition, it would disrupt the biofilm and set the stage for a successful local antibiotherapy. The addition of an antibacterial agent, slowly released into the sulcus, and at a concentration of 100 to 1000 times higher than if the same agent was to be taken systemically, was hoped to further reduce the bacterial presence. Furthermore, it allows the recolonization of the site by members of the genera Streptococcus and *Actinomyces*, which are more host compatible and beneficial. Also by reducing the number of microorganisms, one decreases gingival inflammation and pocket depth, hence altering the local environment. Periodontal pathogens are less likely to develop in an environment where the subgingival temperature, anaerobic conditions, iron concentration, have been changed. Ebersole *et al.*[16] suggested that scaling could alter the host's immune response by naturally "vaccinating" the host. In their belief, scaling induces an elevated antibody response to selected species possibly by introducing microorganisms into the underlying tissues. In their opinion, this process produces higher levels of antibody that are effective in combating local infections.

The results of our study show a significantly greater degree of bacterial reduction when Atridox^{TM} is added to SRP in the treatment of patients with generalized severe chronic periodontitis. This was particularly true when looking at bacteria such as *T. forsythia, P. gingivalis* and *T. denticola*. These pathogens are part of the red complex and co-aggregate strongly *in vitro* and one species of the complex may produce growth factors required by another in that complex[16]. These pathogens are usually encountered in pocket depth greater than 4 mm. In 4-6 mm pockets, *T. denticola* was detected at the surface layer of the plaque, while *P. gingivalis* was detected in the layers beneath. In deep pockets, these species co-exist in large numbers, and have been shown to relate very strongly to clinical parameters such as bleeding on probing.

A previous study has demonstrated a reduction in the species of this red complex after SRP[14]. In this study, it was further demonstrated that the addition of Doxycycline 10%, locally released, to the conventional SRP decreases even more the number of these pathogens. It was also noticed an increase in gram positive microorganisms such as *Actinomyces viscous* and *Streptococcus oralis*. These species are indigenous, host-compatible organisms, and compatible with gingival health[17]. Furthermore, the addition of local chemotherapy seems to help shift the subgingival microbiota to one that was more conductive to periodontal health. Investigators have reported up to 9 months follow up after treatment for clinical results when comparing Atridox™ placement versus SRP, and 6 months follow up when looking at the microbial response[8]. In the study, the authors were comparing the effect of locally applied Atridox™ (alone) to SRP in patients with periodontitis. The clinical results showed that Atridox™ alone was as effective as SRP in reducing pocket depth and gaining attachment levels, whereas the microbiological analysis showed a substantial reduction in anaerobic gram negative bacilli. Also, the authors reported that the local delivery of doxycycline did not change the proportion of naturally doxycycline resistant bacteria present (such as *Streptococci*) and did not increase the acquisition of doxycycline or multi-antibiotic resistance in the subgingival plaque. This being the fortunate result of achieving drug concentrations that are 100 to 1000 times higher in the gingival sulcus when one uses local chemotherapy versus systemic administration. It is unlikely that bacteria survive such high levels of doxycycline that are present in the sulcus for about one week. Especially, if the bio-film that can exert a protective effect by slowing down drug penetration[18,19] has been disrupted by initial SRP of the site, as it has been done in this study.

Conclusion

The topical application of doxycycline 10% has the potential to improve the results of SRP alone with regards to bacterial count, as well as possibly altering the composition of the subgingival plaque (reduction of periodontal pathogens, such as *Tannerella forsythia, Porphyromonas gingivalis* and *Treponema denticola*).

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تأثير تجريف جذور الأسنان مع الدوكسيسايكلين مقارنة بتجريف جذور الأسنان فقط على أنواع البكتيريا المصاحبة لالتهابات اللثة المزمنة

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المستخلص. هدف هذه الدراسة هو معرفة تأثير الحقن الموضعي للدوكسيسايكلين ١٠٪ (اتريدوكس) بعد تجريف جذور الأسنان مقارنة بتجريف جذور الأسنان فقط على تكوين البكتيريا الموجودة بجذور الأسنان لدى المرضى المصابون بالتهاب حاد و مزمن باللثة. باستخدام طريقة تقسيم الفم إلى قسمين: قسم يشمل علاج تجريف جذور الأسنان مع اتريدوكس وقسم آخر يشمل علاج تجريف جذور الأسنان فقط، عينات البكتيريا في جذور الأسنان أخذت في الأساس قبل بدء العلاج وبعد شهرين من العلاج. التحليل الميكروبيولوجي لأنواع البكتيريا نفذت باستخدام تقنية لوحة البحث للحمض النووي. تم فحص العينات الجرثومية لاكتشاف التالي من البكتيريا: *Actinomyces naeslundii*، *Tannerella forsythia*، *Campylobacter conscisus*، *Campylobacter curva*، *Campylobacte rectus*، *Capnocytophaga sputigena*، *Eikenella corrodens*، *Fusobacterium nucleatum*، *Porpyromonas gingivalis*، *Prevotella intermedia*، *Prevotella nigrescens*، *Streptococcus oralis*، *Treponema denticola*، *Aggregatibacter actinomycetemcomitans*. بعد شهرين من العلاج،

كان هناك انخفاض ملحوظ في إجمالي البكتيريا فى كلا القسمين من الفم. و أنواع البكتيريا الضارة باللثة *Tannerella forsythia*، *Porphyromonas gingivalis*، *Treponema denticola* انخفضت إلى حد كبير، بينما ارتفع مستوى بكتيريا *Actinomyces naeslundii* و *Streptococcus oralis* المفيدة للثة عندما تم استخدام علاج الاتريدوكس مع تجريف جذور الأسنان مقارنة بعلاج تجريف جذور الأسنان فقط. تدل نتائج هذه الدراسة على أن الاستخدام الموضعي ١٠٪ دوكسيسيكلين كعامل مساعد لتجريف جذور الأسنان يعتبر علاجًا غير جراحي فعال للمرضى المصابين بالتهاب حاد ومزمن باللثة.