

## Clinical and microbial evaluation of dental scaling associated with subgingival minocycline in chronic periodontitis subjects

### *Avaliação clínica e microbiana da raspagem dental associada à minociclina subgengival em indivíduos com periodontite crônica*

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**ABSTRACT:** The aims of this double-blind randomized clinical trial were to evaluate the presence of periodontal pathogens and the clinical response of periodontal pockets treatment to scaling and root planing (SRP) associated with subgingival minocycline (SM). A total of 36 subjects, 26 to 60 years old ( $40.7 \pm 9.1$ ), who had been previously diagnosed with chronic periodontitis, were included in the present study. Eighteen subjects were selected for the test group (TG), who were treated with SRP plus SM (new treatment), and 18 subjects for the control group (CG) who received SRP plus vehicle (current treatment). Two homologous sites in each subject with a probing depth (PD)  $\geq 6$  mm were chosen. To evaluate the clinical response after treatment, PD was measured at baseline and at 90 days. Microbiological evaluation was performed to detect 7 periodontal pathogens using polymerase chain reaction at baseline, 30, and 120 days. A mean reduction in PD of 2.8 and 2.1 mm was observed in the TG and CG, respectively. At baseline, *P. gingivalis* was the most prevalent organism in both test (65.8%) and control (48.6%) groups. After 120 days it fell to 30.8% in TG and to 23.1% in CG. There were no statistically significant differences between the test and control groups concerning PD ( $p > 0.05$  by Wilcoxon test) or presence of periodontal pathogens ( $p > 0.05$  by Wilcoxon and chi-square;  $p > 0.01$  by Signal test). The results observed showed that the new treatment was as effective as the current treatment in reducing periodontal pathogens and PD among chronic periodontitis subjects.

**DESCRIPTORS:** Bacteria; Dental scaling; Minocycline.

**RESUMO:** O objetivo deste estudo randomizado duplo-cego foi avaliar a presença de periodontopatógenos e o comportamento clínico de bolsas periodontais tratadas com raspagem e aplainamento radicular (RAR) associado à minociclina subgengival (MS). Foram incluídos no presente estudo 36 indivíduos de 26 a 60 anos de idade ( $40,7 \pm 9,1$ ), previamente diagnosticados com periodontite crônica. Dezoito indivíduos foram selecionados para o grupo teste (GT), tratado com RAR e MS (novo tratamento), e 18 indivíduos, para o grupo controle (GC), que recebeu RAR e veículo (tratamento convencional). Foram selecionados dois sítios homólogos em cada indivíduo com profundidade de sondagem (PS)  $\geq 6$  mm para testar a hipótese proposta. Para avaliar o comportamento clínico após o tratamento, a mensuração da PS foi realizada inicialmente e após 90 dias. A avaliação microbiana foi realizada para detecção de 7 periodontopatógenos através de reação em cadeia da polimerase, inicialmente e após 30 e 120 dias. Observou-se redução média de 2,8 e 2,1 mm na PS nos grupos teste e controle, respectivamente. Inicialmente, *P. gingivalis* foi o microrganismo mais prevalente tanto no GT (65,8%) quanto no GC (48,6%). Após 120 dias, houve redução para 30,8% no GT e 23,1% no GC. Não houve diferenças estatisticamente significativas entre os grupos em relação à PS (teste Wilcoxon -  $p > 0,05$ ) e presença dos periodontopatógenos (teste Wilcoxon e qui-quadrado -  $p > 0,05$  e teste do sinal -  $p > 0,01$ ). Os resultados observados demonstraram que o novo tratamento foi tão efetivo quanto o tratamento convencional na redução de periodontopatógenos e de PS em indivíduos com periodontite crônica.

**DESCRIPTORIOS:** Bactérias; Raspagem dentária; Minociclina.

## INTRODUCTION

Periodontitis is a multi-factorial disease with microbial dental plaque as the initiator of peri-

odontal disease. However, the manifestation and progression of periodontitis is influenced by a wide

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variety of determinants and factors including subject characteristics, social and behavioral factors, systemic factors, genetic factors, tooth-level factors, microbial composition of dental plaque and other emerging risk factors<sup>12</sup>.

There is plenty of evidence that over 300 different species of bacteria are recognized in the oral cavity, but only a few are directly associated with periodontal disease<sup>11</sup>. These include *P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans*, *T. forsythensis* (formerly *B. forsythus*), *F. nucleatum*, *Seenomona* species, *C. rectus* and *E. corrodens*<sup>18</sup>.

Mechanical therapy (scaling and root planing) aimed at the removal of pathogens and their products, has been shown to be effective in reducing bacterial colonization to sub-threshold values, in order to control the progression of periodontal disease (current treatment). However, scaling and root planing (SRP) may not eradicate species that have invasive properties, i.e., bacterial species that can reach epithelial cells and subepithelial connective tissues of the periodontium<sup>1</sup>, leading to the use of antimicrobial agents, which may help to eliminate the pathogens<sup>3</sup>.

Several locally delivered antimicrobial agents currently used are designed to enhance the healing process and improve periodontal health. In diseased sites that are more difficult to control, the application of local drug delivery devices may be a valid approach for treating infected sites<sup>9</sup>.

However, research has focused on the development of a new treatment to appropriately deliver the drug into the periodontal pockets. For instance, a method of microencapsulating minocycline hydrochloride in a bioabsorbable polymer (polyglycolide-co-dl-lactide) has been developed, resulting in microspheres that are administered into sites with periodontal disease<sup>22</sup>.

The aim of this study was to evaluate the presence of periodontal pathogens and the clinical response to SRP periodontal pocket treatment associated with subgingival minocycline (SM) in chronic periodontitis subjects.

## MATERIALS AND METHODS

Thirty-six subjects with generalized severe chronic periodontitis were recruited to participate in this double-blind randomized clinical trial, after informed consent (Institutional Committee on Research Involving Human, protocol n. 013/02) was obtained from each subject. To be included in the study, subjects had to meet requirements for

inclusion (non-treated chronic periodontitis, and patient had to be in good general health) and exclusion criteria (according to Cortelli *et al.*<sup>8</sup>, 2003).

At baseline, periodontal and radiographic examinations determined the periodontal status of all subjects, based on the criteria presented by the American Academy of Periodontology<sup>2</sup> (1999). During the periodontal examination, one trained, calibrated examiner measured periodontal probing depth (PD) at 6 sites per tooth, using a manual periodontal probe (PQWBR – Hu Friedy Mfg. Inc. Chicago, IL, USA). The reproducibility of these measurements were analyzed using kappa statistics, and obtained kappa = 0.83. Two homologous sites with PD  $\geq$  6 mm without furcation involvement were selected from each subject to test the hypothesis proposed. After randomization, two groups were formed; one considered a test group (SRP plus SM) and one a control group (SRP plus vehicle). All subjects were examined at baseline and at 90 days for measurement of PD. In addition, a trained examiner evaluated supragingival plaque control (Oral Hygiene Index - OHI<sup>13</sup>) and oral hygiene compliance at baseline and at 90 days.

Subgingival samples were taken from each of the two sites from each subject using sterile paper points (Tanari, Tanariman Industrial Ltda., Manacapuru, Brazil) inserted to the depth of the pockets after the removal of supragingival plaque. Paper points were removed after 15 seconds, and immediately placed in phosphate-buffered saline (Promega, Madison, WI, USA) (pH 7.4) on ice. These microbiological samples were collected at baseline, 30, and 120 days.

The samples in phosphate-buffered saline (pH 7.4) were warmed to 37°C for 10 minutes and were thoroughly mixed on a vortex mixer (PRO Scientific Inc., Oxford, UK); 0.2 ml of the microbial suspension was washed 3 times with distilled water. Then the bacterial pellets were resuspended in 0.1 ml of distilled water, boiled for 10 minutes, and placed on ice. After centrifugation to remove cell debris, the supernatant was used for polymerase chain reaction (PCR) analysis. Briefly, 5  $\mu$ l of the sample was added to 45  $\mu$ l of reaction mixture containing 5  $\mu$ l of 10 X PCR buffer (Promega, Madison, WI, USA), 1.25 unit of Taq DNA polymerase (Promega, Madison, WI, USA), and 0.2 mm of each deoxyribonucleotides (Pharmacia LKB, Piscataway, NJ, USA). PCR amplification was performed using a thermalcycler (Perkin Elmer Cetus, Wellesley, MA, USA). The primer sequences and the PCR procedure were previously described with details by

Avila-Campos *et al.*<sup>4</sup> (1999) (*F. nucleatum*), Saiki *et al.*<sup>14</sup> (1988) (*T. forsythensis*, *C. rectus*, *E. corrodens*, *P. gingivalis* and *P. intermedia*) and Cortelli *et al.*<sup>8</sup> (2003) (*A. actinomycetemcomitans*).

PCR products were analyzed using 1.5% agarose gel (Sigma, Dorset, UK) electrophoresis performed at 4 V/cm in tris-acetate EDTA buffer (Promega, Madison, WI, USA). The gel was stained with 0.5 µg/ml ethidium bromide (Amersham, Arlington Heights, USA) and photographed under 300 nm ultraviolet light.

Each subject received local anesthesia followed by full-mouth SRP both supra- and subgingivally according to individual needs. Following SRP, the test group was treated with minocycline microspheres (Arestin™, OraPharma, Inc., Warmister, PA, USA), while the control group was treated with vehicle (polymer without minocycline - Byofórmula, manipulation pharmacy, São Paulo, Brazil).

The microspheres were dispensed subgingivally to the base of the periodontal pocket by means of a disposable plastic cartridge (OraPharma Inc., Warmister, PA, USA) affixed to a stainless-steel handle. Each cartridge contains 1 mg of minocycline microencapsulated in Poly (glycolic-co dl-lactide) dry powder and it is enough for one periodontal pocket. At 90 days, test and control groups received an additional application of the assigned treatment.

### Statistical analysis

Sample size calculation was performed using the PS – Power Sample Size program (Biostat, Englewood, NJ, USA), version 2.1.30. The sample size of 36 subjects was reached when  $\alpha = 0.05$ , power = 0.80, and an initial difference between mean pocket depth of 0.1 mm among groups, and a 1:1 proportion of subjects in each group were established.

The changes between the baseline and the 90-day measurement of PD for test and control groups were examined by Wilcoxon test ( $p < 0.05$ ). Differences in the OHI scores between groups and exams were analyzed by analysis of variance, Newman-Keuls and HSD – Tukey (Honest Significant Differences) tests ( $p < 0.05$ ). The prevalence of each pathogen at baseline, 30, and 120 days was analyzed by Wilcoxon test ( $p < 0.05$ ). The proportional distribution of the bacteria between test and control groups was analyzed by Signal test ( $p < 0.01$ ). In addition, a chi-square test was used to determine a positive association between two bacterial species ( $p < 0.05$ ).

## RESULTS

Thirty-six subjects (17 female and 19 male), aged 26 to 60 years old (mean  $40.7 \pm 9.1$ ), participated in the present study. Data related to the mean of PD indicates that, at baseline, the values for test and control groups were not different,  $7.8 \pm 0.9$  mm and  $7.9 \pm 0.8$  mm, respectively. After 90 days, the mean of PD decreased to  $5.0 \pm 1.4$  mm among test group, and  $5.8 \pm 2.1$  mm for the control group. The results did not show a statistically significant difference between the test and the control groups ( $p > 0.05$  by Wilcoxon test).

Median values of the OHI scores were 93.5% for the test group and 95.5% for the control group at baseline, and after 90 days it decreased to 67.5% and 72.0% respectively for the test and control groups. A statistically significant difference was observed between baseline and 90 days for the OHI scores within each of the groups. However, when the difference between the test and control groups was investigated, no statistically significant differences were observed between each time point.

The prevalence of analyzed bacteria present in the 36 subjects, for the test and control groups at baseline, 30, and 120 days is indicated in Table 1. In the test group, the prevalence of *A. actinomycetemcomitans* ( $p = 0.01$ ), *P. gingivalis* ( $p = 0.00$ ) and *T. forsythensis* ( $p = 0.02$ ) significantly decreased after 120 days ( $p < 0.05$ ). There was a significant increase in *F. nucleatum* ( $p = 0.01$ ). The prevalence of bacterial species was higher among test than among controls at all three time-points. However, data indicated that prevalence decreased as time increased. At baseline, prevalence of bacteria ranged from 22.9 - 65.8% among the test group, and from 11.5 - 48.6% among controls. At 30 days, the prevalence among the test group ranged from 14.9 - 37.1% and from 14.9% - 33.4% among controls. At 120 days, bacterial prevalence among the test group had increased, compared to the 30-day time point (range: 15.4 - 46.2%). However, the values were still below the levels observed at baseline. Among controls, prevalence at 120 days was lower than at the two previous time-points, ranging from 7.7% to 30.8%.

The Signal test did not show significant differences in the proportional distributions of periodontal pathogens between the test and control groups ( $p > 0.01$ ).

The correlation between bacterial species was investigated in test and control groups (Figure 1). Positive associations were found between *A. acti-*

**TABLE 1** - Prevalence (%) and distribution of periodontal pathogens among test and control groups (n = 36).

Bacteria	Time	Baseline		30 days		120 days	
		Test	Control	Test	Control	Test	Control
<i>A. actinomycetemcomitans</i>		34.3	20.0	33.4	18.6	15.4*	15.4
<i>P. gingivalis</i>		65.8	48.6	37.1*	33.4*	30.8*	23.1*
<i>P. intermedia</i>		28.6	20.0	22.3	29.7*	23.1	15.4
<i>T. forsythensis</i>		31.5	28.6	22.3	26.0*	15.4*	7.7*
<i>C. rectus</i>		25.8	11.5	22.3	14.9	15.4	15.4
<i>E. corrodens</i>		22.9	20.0	14.9*	33.4	30.8	23.1
<i>F. nucleatum</i>		22.9	31.5	14.9*	18.6*	46.2*	30.8

\*Statistically significant difference (p < 0.05) – Wilcoxon test.

**FIGURE 1** - Chi-square test of associations among examined species in test and control groups at baseline, 30, and 120 days.

Microorganism	Aa	Pg	Pi	Cr	Tf	Ec
Pg	NI - TG					
	NI - CG					
Pi	NI - TG					
		NI - CG				
Cr	NI - TG	NI - CG				
Tf		NI - CG		NI - CG		
Ec						
		NI - TG/CG				
Fn						

NI = not-independent; TG = Test group; CG = Control group. Aa = *A. actinomycetemcomitans*; Pg = *P. gingivalis*; Pi = *P. intermedia*; Cr = *C. rectus*; Fn = *F. nucleatum*; Ec = *E. corrodens*; Tf = *T. forsythensis*.

*actinomycetemcomitans* and *P. intermedia* (p = 0.0049,  $\chi^2 = 7.93$ ), *A. actinomycetemcomitans* and *C. rectus* (p = 0.0176,  $\chi^2 = 5.64$ ), *A. actinomycetemcomitans* and *P. gingivalis* (p = 0.0485,  $\chi^2 = 3.89$ ), and *A. actinomycetemcomitans* and *E. corrodens* (p = 0.0211,  $\chi^2 = 5.32$ ), all in the test group.

Among controls, statistically significant positive associations were found between *P. gingivalis* and *C. rectus* (p = 0.0288,  $\chi^2 = 4.78$  and p = 0.0050,  $\chi^2 = 7.88$  at baseline and at 120 days, respectively); *P. gingivalis* and *T. forsythen-*

*sis* (p = 0.0186,  $\chi^2 = 5.54$ ); *C. rectus* and *T. forsythensis* (p = 0.0290,  $\chi^2 = 4.77$ ); *P. gingivalis* and *P. intermedia* (p = 0.0370,  $\chi^2 = 4.35$ ); *A. actinomycetemcomitans* and *P. gingivalis* (p = 0.0050,  $\chi^2 = 7.88$ ); and *A. actinomycetemcomitans* and *C. rectus* (p = 0.0003,  $\chi^2 = 13.00$ ).

## DISCUSSION

Mechanical therapy is usually the first mode of treatment recommended for most periodontal

infections, as the mean probing depth reduction ranges from 0.03 in pocket depths between 1-3 mm, to 2.16 mm in pocket depths greater than 6 mm<sup>7</sup>. In scientific investigations, probing depth is a method frequently used to evaluate both treatment and disease progression<sup>21</sup>. Badersten *et al.*<sup>5</sup> (1984) showed a reduction in probing depth of approximately 2.3 mm in a study of nonsurgical therapy of subjects with advanced chronic periodontitis. The results of this study are in agreement with the previous literature, as subjects in both groups responded favorably to initial treatment, with a reduction in PD of 2.9 mm among test and 2.4 mm among control groups.

In the present study, there were no significant differences between test and control groups with regard to probing depth. Likewise, Timmerman *et al.*<sup>19</sup> (1996) showed no additional benefit in probing depth when SRP was associated with SM, compared to controls.

It has been shown that the root surface itself harbors viable bacteria that may provide niches for subgingival bacterial recolonization<sup>1,10</sup>. The root may become a bacterial reservoir from which periodontal pathogenic bacteria can recolonize previously treated pockets and contribute to the failure of therapy or the recurrence of the disease<sup>6</sup>. The present study analyzed the presence of periodontal pathogens at 30 and 120 days, time points established on the attempt to evaluate possible microbial recolonization, always 30 days after the subgingival administration of minocycline. Sbordone *et al.*<sup>15</sup> (1990) suggested that the microbiota might reestablish itself within 40-60 days following subgingival debridement with poor supragingival plaque control. Deep pockets are particularly difficult to control, since recolonization of the subgingival microbiota occurs at around 120-240 days, despite meticulous supragingival plaque control and multiple sessions of subgingival debridement<sup>9</sup>. The subjects enrolled in our study received oral hygiene instructions and were closely followed by a trained examiner who evaluated plaque control and oral hygiene compliance. Despite the close plaque control, some of the severely periodontally-involved participants of the present study showed a similar pattern of recolonization previously observed by other investigators.

*P. gingivalis* in our study was the most prevalent bacteria both in the test (65.8%) and control (48.6%) groups at baseline. This bacteria was found in a high percentage in the test and control groups after 30 (37.1% and 33.4%, respectively)

and 120 days (30.8% and 23.1%, respectively). *P. gingivalis* has been considered as one of the most important pathogens in advanced periodontitis based on its high prevalence in progressive periodontitis lesions<sup>16,17</sup>. After 30 days, the prevalence of only *P. intermedia*, *C. rectus* and *E. corrodens* increased in the control group. A reduction in bacterial prevalence was observed in all species except for *C. rectus*, *E. corrodens* and *F. nucleatum*, which did not decrease after 120 days. The PCR method was used to characterize not only the specific bacterial types, but also the possible intercorrelations that may occur. The results of the present study confirm that the studied bacterial correlations do not follow a unique pattern. These results may be of use in the explanation of bacterial interassociation, one of the objectives of molecular biology. The present study also demonstrated no significant statistical differences between the test and control groups with regard to microbiological parameters. The high sensitivity of PCR may partially explain these findings, and the differences in the microbial profiles could be observed if a quantitative microbiological instead of a PCR approach had been chosen. Similarly, Timmerman *et al.*<sup>19</sup> (1996) showed a significant reduction in the prevalence of periodontal pathogens over a 15-month period, but no significant reduction was observed when local delivery of minocycline was used.

In contrast, van Steenberghe *et al.*<sup>20</sup> (1999) demonstrated that SM as an adjunct to SRP resulted in the reduction of *P. gingivalis*, *P. intermedia*, *C. rectus*, *E. corrodens*, *F. nucleatum* and *T. denticola*, a reduction in PD greater than that seen in the control. Although this study protocol<sup>20</sup> did not allow optimal oral hygiene instruction and reinforcement, this was emphasized in the present study.

Williams *et al.*<sup>22</sup> (2001) reported that SRP plus SM was effective in reducing PD in chronic periodontitis individuals as shown in the present study. However, their results demonstrated that adjunctive therapy with minocycline microspheres provided significantly greater PD reduction than SRP alone.

In the past 15 years, there has been an emergence of a range of adjunctive antimicrobial regimens designed to aid the mechanical methods of dealing with subgingival plaque. To achieve a reduction or elimination of pathogens, a subgingival application of antiseptics and/or antibiotics has often been considered, but subjects enrolled in the present study did not benefit from local delivery of

minocycline. However, subgingival debridement in combination with oral hygiene instruction by itself has been shown to be an effective treatment.

## CONCLUSION

In conclusion, the results of this double-blind randomized clinical trial indicated that SRP associated with SM (new treatment) was as effective as mechanical therapy alone (current treatment) in reducing both the prevalence of periodontal patho-

gens and probing depth in chronic periodontitis subjects.

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