SUBGINGIVALLY APPLIED MINOCYCLINE MICROGRANULES IN SUBJECTS WITH CHRONIC PERIODONTITIS. A RANDOMIZED CLINICAL AND MICROBIOLOGICAL TRIAL

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ABSTRACT

The aim of this study was to evaluate clinical and microbiological effects of subgingival minocycline microgranules when used as an adjunct to scaling and root planing in subjects with Chronic periodontitis.

Twenty-six non-smoker volunteers participated in the study. Four opposite sites, clinically standardized, with bleeding on probing (BOP) and pocket depth (PD) ≥ 6 mm were selected. Baseline BOP, PD and Clinical attachment level (CAL) were measured and microbiological samples were collected from the study sites and analyzed using PCR. Porphyromonas gingivalis (Pg) Tannerella forsythia (Tf), Treponema denticola (Td) and Aggregatibacter actinomycetemcomitans (Aa) were detected. One side of the mouth was randomly allocated to the experimental treatment: scaling and root planing plus minocycline microgranules (Test group= T) and the other side of the mouth to scaling and root planing alone (Control group= C). At days 30 and 90, clinical and microbiological examination was repeated.

After 30 days BOP was reduced to 81% in C and to 12% in T and at day 90 to 58% in C and to 8% in T (p< 0.05). PD was significantly reduced in both groups (C: 4.8mm, T: 4.2mm) favoring T at days 30 and 90 (p< 0.05). CAL reduction at day 30 showed no difference between groups. At day 90, CAL reduction was higher in T (p< 0.05). At days 30 and 90 Pg, Tf, Td and Aa was reduced in both groups. Pg reduction was significantly greater in group T. At day 90 frequency of sites with Td decreased in T and increased in C (p< 0.05).

No adverse effect was observed.

This study showed that minocycline microgranules adjunct to scaling and root planing resulted in grater reduction of BOP and PD, higher CAL gain, increased probability of Pg suppression and retarded recolonization of Td than root instrumentation alone.

Key words: *Periodontitis, Minocycline, Porphyromonas gingivalis, drug delivery systems.*

MICROGRÁNULOS DE MINOCICLINA SUBGINGIVAL EN SUJETOS CON PERIODONTITIS CRÓNICA. ESTUDIO CLÍNICO Y MICROBIOLÓGICO ALEATORIZADO

El objetivo de este estudio fue evaluar el efecto clínico y microbiológico de microgránulos de Minociclina, colocados subgingivalmente como coadyuvante del raspaje y alisado radicular en pacientes con Periodontitis crónica severa.

Participaron 26 sujetos voluntarios con Periodontitis crónica, no fumadores. Se seleccionaron 4 sitios contralaterales con Sangrado al Sondaje (SS) y Profundidad al Sondaje (PS) ≥ 6 mm. Condición Basal (CB): se registró SS, PS y Nivel de Inserción (NI). Se determinó mediante PCR presencia de Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf), Treponema denticola (Td) y Aggregatibacter actinomycetemcomitans (Aa). Un lado de la boca fue aleatoriamente asignado al tratamiento experimental: grupo T, el otro al tratamiento control: grupo C.

Al día 30 y 90 se repitieron los exámenes clínicos y microbiológicos. Día 30: el SS se redujo al 81% en el grupo C y al 12% en el grupo T (p<0,05).Estas diferencias se mantuvieron al día 90 (C: 58%, T: 8%) (p<0.05).Día 30 y 90: hubo disminución de la PS en ambos grupos, siendo significativamente mayor en el grupo T (p<0,05).En ambos grupos hubo disminución significativa del NI (p<0,05), no hubo diferencias entre los grupos al día 30 y sí al día 90. A los 30 y 90 días en ambos grupos se redujo la prevalencia para Pg, Tf ,Td y Aa. A los 30 y 90 días la reducción de sitios con Pg fue mayor en el grupo T (p=0,002). A los 90 día Td disminuyó en el grupo T y aumentó en el grupo C (p=0,023).No se observaron efectos adversos.

Los resultados mostraron que la aplicación subgingival de microgránulos de minociclina adjunta al raspaje y alisado radicular produjo una reducción mayor del SS, la PS y el NI que el raspaje y alisado solo,aumentó la probabilidad de suprimir Pg y retardó la recolonización con Td.

Palabras clave: *Periodontitis, Minociclina, Porphyromonas gingivalis, sistema de aplicación de fármacos.*

INTRODUCTION

The role of certain bacteria residing in the subgingival biofilm, such as P*orphyromonas gingivalis, Tannerella forsythia, Aggregatibacter actinomycetemcomitans* and *Treponema denticola,* in the development and pathogenesis of periodontitis is well established [1-3].

Conventional periodontal mechanical therapy without adjunctive chemotherapy is often sufficient to suppress bacterial pathogens and attain periodontal health [4,5]. Adjunctive local or systemic chemotherapies can be used to try to enhance results or improve outcomes at sites not responsive to conventional therapy [6].

Several studies have indicated the effectiveness of systemic antibiotics adjunctive to scaling and root planing, particularly in terms of pocket depth reduction and attachment level gain, not only in Aggressive periodontitis, but also in advanced chronic periodontitis[7,8]. Inherent to systemic administration of antibiotics are problems such as the risk of increasing bacterial resistance as well as encountering potentially unpleasant side effects and problems with patient compliance.

As an alternative to systemic therapy, local delivery of antibiotics into periodontal pockets has been suggested. Local drug administration can avoid many of the side effects associated with systemic antibiotic therapy by limiting the agent to the periodontal pocket [9,10]. When only few sites in the mouth are affected, local delivery may be especially valuable.

Minocycline HCL is a semi-synthetic tetracycline, effective against *Porphyromonas gingivalis, Prevotella intermedia, Eikenella corrodens, Campylobacter rectus* and *Aggregatibacter actinomycetemcomitans* [11-13]. In addition to its antibacterial properties, it is inhibitory for collagenase, produced by *P. gingivalis*, neutrophils and fibroblasts from periodontal tissues [14]. Like all tetracyclines, minocycline exhibits substantivity, adsorption and subsequent desorption from dentine, while maintaining antimicrobial activity [15].

The first studies on local administration of minocycline used an ***ointment*** **(Periocline® - Cynamid®)**. Satomi et al [16] evaluated the concentration in gingival fluid of this minocycline ointment, and found 1000 ug-ml one hour after subgingival administration, which is 100 times the MIC90 required for periodontal pathogens. Concentrations in gingival fluid over 100 ug- ml were maintained over the first 6 hours. After 72 hr. 3.4 ug / ml of minocycline were found. This concentration exceeds the MIC 90 for *T.denticola, A. actinomycetemcomitans, P.gingivalis, P. intermedia* and *F. nucleatum* [13]. In clinical studies the addition of this ointment in three or four sessions after scaling and root planing, showed benefits in the reduction of deep pockets in a 3-month period [17-19] and in a 15-month period [20]. Microbiologically, Nakagawa et al. [17] found grater reduction of Gram-negative periodontopathic bacteria and van Steenberghe et al. [18,20] reported greater reduction of *P. gingivalis* and *P. intermedia.*

A ***gel*** **(Dentomycin®)** failed to provide additional benefits to the effect of scaling and root planing alone. Graca et al.[21] studied a small number of patients, Kinane & Radvar[22] evaluated residual pockets, McColl et al.[23] applied the gel during the maintenance phase, Timmerman et al.[24] found benefits at 1 and 3 months but not at 18 months, Jain et al.[25] found benefit only in pocket depth reduction at 6 and 9 months.

The third product developed for sustained release of minocycline is a ***dry powder*** **(*microspheres*) (Arestin®).** It was evaluated as an adjunct to scaling and root planing in numerous studies[26-33] for periodontal maintenance[34] and treatment of peri-implant disease[35] with, clinical and microbiological benefits.

The aim of this study was to evaluate the clinical and microbiological effect of ***minocycline microgranules*** applied subgingivally as an adjunct to scaling and root planing, in deep periodontal pockets of patients with Chronic periodontitis.

MATERIALS AND METHODS

Study design

This study was designed as a randomized clinical-microbiological trial, split mouth, single-blind with 3-month follow- up. The protocol was approved by the Ethics Committee of Maimonides University and the study was conducted according to the principles outlined in the Declaration of Helsinski on experimentation involving human subjects. Participants were volunteers and signed informed consent at enrollment.

Subject population

Subjects from the Periodontics Service of the Commercial Workers Union, Quilmes, Buenos

Aires were recruited voluntarily. Subjects were included if they were over 35 years of age, non-smokers, and showed clinical and radiographic evidence of moderate or severe Chronic periodontitis according to the criteria described by Armitage [36], and had at least 2 non- contiguous sites with probing pocket depth ≥ to 6 mm and ≤ to 8mm in each side of the mouth. Subjects were excluded from the study if they required antibiotic pre-medication for the performance of periodontal examination and treatment, suffered from any other systemic diseases (cardiovascular or diabetes), were pregnant or lactating female, had received antibiotic treatment in the previous 3 months, were taking long-term anti-inflammatory drugs, were allergic to minocycline, were not able to provide consent to participate in the study, or did not accept the proposed treatment plan.

Experimental design (Fig.1)

Pre-experimental: A clinical-radiographic examination was carried out to determine the periodontal conditions of the subjects. Thirty-two patients who met the inclusion criteria were enrolled in this study. Subjects went through motivation sessions and received instructions in oral hygiene. Two non-contiguous sites on each side of the mouth, with probing pocket depth ≥ to 6 mm and ≤ to 8mm and bleeding on probing were chosen per subject, excluding teeth with furcation lesions. During this phase full- mouth scaling and root planing was carried out, except for the selected sites.

Treatment: After baseline examination, every two sites per patient were randomly assigned to receive one or the other of the following treatments: control treatment: only scaling and root planning, or experimental: scaling and root planing and subgingival application of minocycline microgranules.

Each microgranule contained 0.35 mg minocycline hydrochloride incorporated to a bio-resorbable polymer (microcrystalline cellulose, sodium croscarmellose, hydroxypropylmethylcellulose phthalate 50, hydroxypropylmethylcellulose, light mineral oil, orange Opaspray). Eight microgranules per site were placed subgingivally, equivalent to 2.8 mg of minocycline. After instrumentation of the experimental sites, the area was isolated with cotton rolls, and a curette moistened with water was used to place the microgranules into each pocket with gentle apical movements (Fig. 2). Patients were instructed not to brush or use any interproximal hygiene implement on the study teeth for 24 hours. The treatment was conducted by one experienced periodontist (C.R.) throughout the study.

Clinical and microbiological examination

At days 30 and 90, clinical examination and microbiological sampling were repeated at the selected sites. After the examination on day 30, professional supragingival prophylaxis was carried out and oral hygiene measures were reinforced. A single trained, calibrated researcher (V.Ch.), to whom the assigned treatment was unknown, examined all the patients clinically and microbiologically.

Clinical examination: Clinical parameters were assessed using a Marquis CP 12(Hu-Friedy) periodontal probe. The following parameters were registered: (I) plaque was recorded by assigning a binary score to each surface (1 for plaque present, 0 for absent) (II) bleeding on probing to the bottom of the pocket, after 15 seconds (BOP): 0 (absent)- 1 (present), (III) probing depth (PD)mm: from the gingival margin to the bottom of the periodontal pocket, (IV) Clinical attachment level (CAL) from the cemento-enamel junction, crown margin or restoration to the bottom of the pocket. Measurements were rounded to the nearest millimeter.

Subgingival sample collection: After the clinical parameters had been recorded, the area was isolated with cotton rolls and supragingival plaque was removed with sterile gauze. Two sterile paper points number 30 or 35 were placed in each site for 15 seconds and immediately placed in separate Eppendorf tubes containing 200 µl of phosphate-buffered sterile saline.

Microbiological procedures: To amplify the bacterial ADN from A*ggregatibacter actinomycetemcomitans* (Aa)*, Tannerella forsythia* (Tf), and *Porphyromonas gingivalis* (Pg), a Multiplex PCR was carried out. Specific forward primers for each bacteria and a conserved reverse primer were used, following Tran & Rudney [37] (Table 1).

PCRs were carried at a final volume of 31µl. The reaction mixture consisted of 18 µl molecular biology quality water, 4 µ sample, 0.5 µl primer *Aa* forward 50 nM, 0.8 µl primer *Pg* forward 50 nM, 1 µl primer *Tf* forward 50nM, 0.5 µl conserved reverse primer 50nM, 0.125µl dNTPs 10 nM, 1 U Taq DNA polymerase (Taq-Free, Inbio-Highway), 5µl Buffer Green 5 X (Promega) and 1 µl Cl_2Mg 25

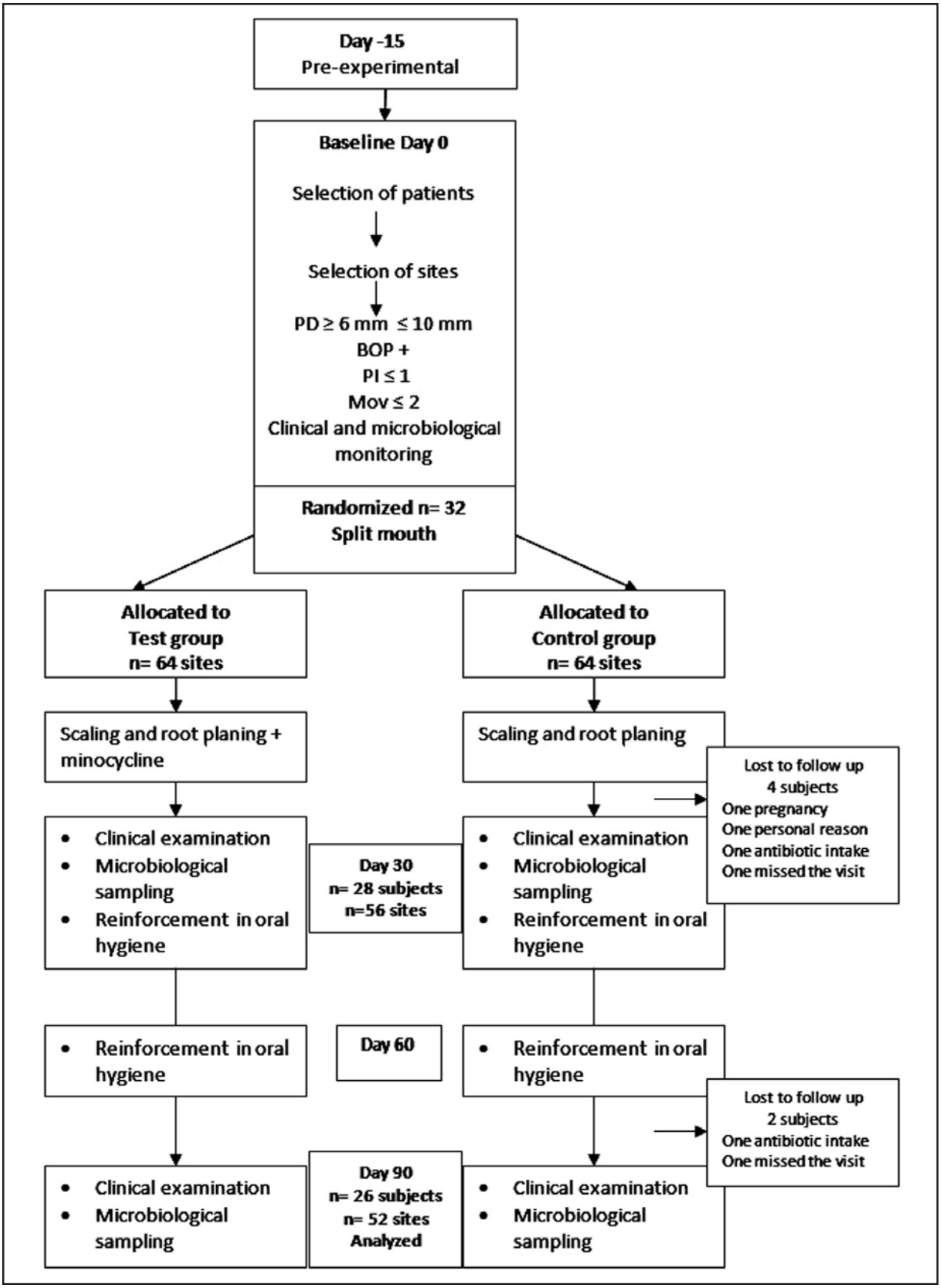

Fig. 1: Study design.

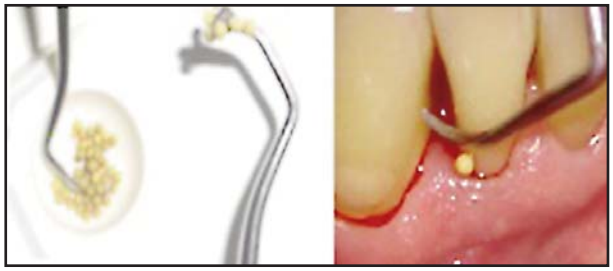

Fig. 2: Clinical application of minocycline microgranules.

mM. The expected product lengths were 360 bp for *Aa*, 745 b for *Tf* and 197 bp for *Pg*.

For detection of *T. denticola (Td)* forward and primer reverse was utilized according to Ashimoto [38], obtaining an amplicon of 300 pb. The reaction mixture, with a final volume of 29 µl, consisted of 18 µ molecular biological quality water, 4 µl sample, 1 µl forward + reverse primer 50 nM, 0.125 µl dNTPs 10 mM, 1 U Taq DNA polymerase (Inbio-Highway),

5 µl reaction Buffer Green 5 X (Promega) and 1 µl Cl_2Mg 25 mM.

The cycling conditions consisted of 35 cycles (1 minute at 94°C, 1 minute at 61°C and 1 minute at 74° C) and an initial denaturation step of 15 minutes at 94°C preceded the amplification cycles. Amplification products were separated in agarose gels (Invitrogen) with red gel coloring (Genbiotech). The gels were observed under light translumination. Amplicones lengths were confirmed by comparing with a molecular weight marker (100pb DNA Ladder, Genbiotech).

Statistical analysis

For the analysis of PD and CAL data, analysis of variance (ANOVA) was applied, through a repeated measures design. PD and CAL data from both sites per subject receiving the same treatment were averaged. The Mauchly test was applied to study the sphericity assumption. The normality of the residuals was studied by the Shapiro-Wilks test. Post hoc pairwise comparisons between groups were carried out through the Tukey test.

The McNemar test was used to compare presence of BOP and of pathogens. The presence of BOP or of the pathogen at one of the two sites on the same side of the mouth (same group), was considered as a positive result. Tests with $p < 0.05$ were considered significant. All statistical analyses were performed using InfoStat software (version 2013, Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina).

RESULTS

Of the 32 subjects initially enrolled, 26 completed the study (11 males, 15 females; age range: 39-56 years; mean: 45.6 years). Eight subjects were lost to follow-up due to reasons unrelated to the study and were not included in the analysis (Fig.2).

Clinical parameters

At initial examination no statistically significant difference was observed among groups for any of the parameters evaluated ($P>0.05$).

Bleeding on probing (Table 2): 100% of the sites of both groups bled on probing at baseline. At day 30 after treatment, this percentage was reduced to 81% in the control group and 12% in the test group. Reductions in the percentage of sites that bled on probing at days 30 and 90 were statistically significant in favor of the test group ($p < 0.05$).

Probing pocket depth (Table 3): Pocket depth showed no differences between the test and control groups at baseline. At day 30 both groups showed statistically significant probing depth reduction when compared to the respective baseline values ($p < 0.05$), however, the test group showed significantly greater improvement than the control group at days 30 and 90 ($p < 0.05$).

Clinical attachment level (Table 4): In both groups a significant reduction ($p<0.05$) was observed at day 30, with no significant difference between groups. At day 90 differences favoring the Test group were found ($p < 0.05$).

Microbiological results (Table 5)

Baseline data showed no difference between groups regarding the presence of the four pathogens studied: more than 70% of the subjects in both groups were positive for *Pg, Tf* and *Td,* detection frequency of *Aa* was less than 40% in both groups.

At 30 and 90 days the percentage of sites positive for Pg was reduced in both groups, with the reduction being significantly greater in the experimental group.

The percentage of positive sites for *Tf, Aa* and *Td* was reduced at day 30 in both the control group and in the experimental group, and no significant differences was found at this time. At day 90, no

Table 1: Primers used for amplification of gene ARNr 16S *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, *T. denticola*.

Bacteria	Primer	Amplicon
A. actinomycetemcomitans (Aa)	5'-TACAGGGGAATAAAATGAGATACG-3'	360 pb
P. gingivalis (Pg)	5'-ATTGGGGTTTAGCCCTGGTG-3'	197 pb
T. forsythia (Tf)	5'-TACAGGGGAATAAAATGAGAT CG-3'	745 pb
Reverse (Aa, Pg, Tf,)	5'-ACG TCA TCC CCA CCT TCC TC-3'	
T. denticola (Td)	5'-TAA TACCGAATGTGCTACTTTACAT-3'	300 pb
T. denticola (Td)	5´-TCAAAGAAGCATTCCCTCTTCTTCTTA-3'	

Table 2: Bleeding on probing (BOP). Change in the frequency of bleeding sites from day 0 to day 90.

Day 0		Day 30		Day 90	
Control	**Test**	**Control**	**Test**	**Control**	**Test**
26 (100%)	26 (100%)	21 (81%)	3 (12%)	15 (58%)	2 (8%)
p > 0.20		p = 0.0001		p = 0.0001	

BOP was positive when present in at least one of the study sites on the same side (group) of the mouth. n=26 subjects.

Table 3: Probing pocket depth (PD) (mm) at Baseline, 30 and 90 days.

		Day 0		Day 30		Day 90	
Group	**n**	**Mean**	**S.E.**	**Mean**	**S.E.**	**Mean**	**S.E.**
Control	26	6.3^{A}	0.08	4.8^{B}	0.22	4.7^{B}	0.24
Test	26	6.6^{A}	0.10	4.2^{C}	0.19	3.8^{C}	0.21

S.E: Standard error. Different letters indicate significant difference between groups ($p < 0.05$)

Table 4: Clinical attachment level (CAL) (mm) at Baseline, 30 and 90 days.

		Day 0		Day 30		Day 90	
Group	**n**	**Mean**	**S.E.**	**Mean**	**S.E.**	**Mean**	**S.E.**
Control	26	6.1^{A}	0.65	5.4^{B}	0.89	5.1^{B}	0.96
Test	26	6.5^{A}	0.78	5.1^{BC}	1.14	4.8^{C}	1.12

S.E.: Standard error Different letters indicate significant differences ($p < 0.05$)

Table 5: Number and percentage of positive sites for the pathogens studied at 0, 30 and 90 days.

	Day 0			Day 30			Day 90		
Pathogen	**Control**	**Test**	**p**	**Control**	**Test**	**p**	**Control**	**Test**	**p**
Pg	26 (100%)	26 (100%)	>0.20	16 (62%)	3 (12%)	0.002	13 (50%)	1 (4%)	0.002
Aa	8 (31%)	10 (38%)	>0.20	6 (23%)	7 (27%)	>0.20	3 (12%)	5 (19%)	>0.20
Tf	19 (73%)	20 (77%)	>0.20	1 (4%)	2 (8%)	>0.20	1 (4%)	0 (0%)	>0.20
Td	24 (92%)	25 (96%)	>0.20	11 (42%)	11 (42%)	>0.20	17 (65%)	8 (31%)	0.023

Pathogen presence was positive when present in at least one of the two study sites on the same side (group) of the mouth.
P. gingivalis (Pg), T forsythia (Tf), A actinomycetemcomitans (Aa), T denticola (Td).

differences was found for *Tf* and *Aa* however, the percentage of *Td* positive sites decreased in the Test group between day 30 and day 90 and increased in the control group,thus the difference between groups was significant .

Percentage of sites with clinically and microbiologically relevant changes (Table 6)

Both treatments caused a significant reduction in deep pockets, increasing the number of shallow sites. The percentage of pockets that were reduced after treatment from ≥ 6 mm to ≤ 4 mm was significantly greater in the Test group (65.4 % vs 46.2 %), and the number of remaining deep sites (≥ 5mm) was greater in the Control group (53.8% vs 34.6%). Residual pockets (≥ 5 mm) in the Control group showed bleeding (19/28) more frequently than in the test group (1/18). *P. gingivalis* was found in residual pockets of the Control group (15/28) and not detected in the Test group (0/18).

DISCUSSION

The development of local controlled release systems has allowed the use of low dosage of antimicrobials in localized sites of periodontal destruction, reaching high concentrations, and reducing the risk of adverse effects and possible bacterial resistance[9].

In periodontics, minocycline is more effective for local, rather than systemic use. Even though periodontal pathogens are sensitive to minocycline in vitro, when it was orally administered, the concentration in gingival fluid did not show predictable concentrations [39].

Numerous studies concluded that local administration of 2% minocycline hydrochloride ointment or 1 mg minocycline microspheres in deep periodontal pockets provides clinical and microbiological benefits improving the effect of scaling and planing alone [40,41].

Our findings showed that minocycline microgranules applied along with scaling and root planing, at sites with probing pocket depth between 6 and 8 mm, in non-smoker patients with Chronic periodontitis, produced greater reduction in probing depth, and percentage of bleeding sites, greater attachment gain and greater reduction of *Porphyromonas gingivalis* .

Evidence shows that a favorable clinical response after scaling and root planing is associated with reduction in the species belonging to the red complex: *Pg, Tf* and *Td* [5]. In agreement with these findings, in our study, both groups showed a reduction in the red complex bacteria, with the expected clinical benefit. The differences in the clinical response favoring the test group, considering that *Td* and *Tf* reduction was similar in both groups can be explained because Pg was suppressed at a larger percentage of sites.

This clinical and microbiological correlation can better be understood by analyzing the data according to the percentage of closed pockets post treatment, 66% in the test vs 36.5 % in the control group, difference that was maintained at day 90 in accordance with the sustained absence of *Pg.* This beneficial ecological conditions achieved in

Table 6: Number /Percentage of sites with relevant clinical and microbiological changes at 30 and 90 days: Pockets changing from ≥ 6 mm at baseline to ≤ 4 mm after treatment and residual pockets ≥ 5 mm.

	Day 30			Day 90		
	Control	Test	p	Control	Test	p
n of initial sites ≥ PD 6 mm	52	52		52	52	
n (%) of sites changing from ≥ 6 mm at baseline to ≤ 4 mm after treatment *	19 (36.5%)	32 (66%)	<0.05	24 (46.2%)	34 (65.4%)	< 0.05
*Pg positive sites	4/19	1/32		2/24	1/34	
Percentage residual pockets ≥ 5mm ∞	33 (63.5%)	20 (34%)	< 0.05	28 (53.8%)	18 (34.6%)	< 0.05
∞ BOP positive sites	23/33	2/20		19/28	1/18	
∞ Pg positive sites	18/33	3/20		15/28	0/18	

the minocycline group at the immediate post-treatment, can probably explain the observed delay in recolonization of *Td,* compared with the rebound in the Control groups.

It is difficult to compare our results with other studies, because besides the difference in the pharmaceutical form of the drug there are also differences in the concentration, frequency of application and in the study periods.

Other products containing minocycline applied lower doses: Periocline® (2% ointment) and Dentomycin® (2% gel) provide 0.5 to 1 mg of minocycline per site (25 to 50 mg of ointment or gel is placed in each pocket, of the product that contains 10 mg of minocycline in 500 mg of vehicle). Arestin® cartridge (microespheres) contains 1mg of minocycline and 3 mg of powder vehicle which is utilized to fill only one pocket. In our study 8 microgranules were placed per site, equivalent to 2.5 mg-3 mg per pocket.

Despite this variability, our microbiological results in relation to Pg suppression, are consistent with the results of other studies using Arestin® [26,30] or Periocline® [18,20]. The clinical results are also consistent, many studies report a larger number of sites with probing depth ≤ 4 mm and absence of bleeding after adjunctive minocycline treatment [17-20,26-33].

No adverse side effect with local minocycline is described in the literature, and no adverse side effect has been found with this alternative form of application.

Our study did not evaluate smokers, but it is worth noting that other authors have found clinical and microbiological benefit with minocycline (Arestin®) in smokers [31].

Benefit achieved with systemic or local antimicrobials adjunct to scaling and root planing has been reported in the literature for deep pockets[42]. Upon considering the clinical relevanceof our results, it should be taken into account that the sites evaluated had pockets between 6 and 8mm at baseline.

This study proposes ***microgranules*** of minocycline HCL, a new method for subgingival administration of Minocycline. These are 0.8 mm diameter spheres, each containing 0.35 mg of the drug incorporated to microcrystalline cellulose. Due to their size, they can be placed individually in the periodontal pocket using a moistened instrument to which they adhere by surface tension.

In view of the results of this study we can conclude that microgranules could be considered an alternative form for local subgingival application of minocycline, as an adjunt to scaling and root planning, in deep periodontal pockets of patients with Chronic periodontitis.

Further studies should be conducted on periodontal maintenance and the treatment of perimplant disease.

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