

Oral Antiseptic and Periodontitis: A Clinical and Microbiological Study

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Abstract

Purpose: This study evaluated the efficacy of a xanthan-based chlorhexidine gel (Xan-CHX) used as antiseptic in periodontal pockets after scaling and root planing in patients with generalized mild-severe chronic periodontitis.

Materials and Methods: 30 systemically healthy patients, 15 males and 15 females (mean age 54,1 ± 6,9 years), diagnosed for chronic periodontitis were enrolled in this study. For each patient, in the 1st and 4th quadrant was applied antiseptic (Test sites); while the 2nd and 3rd were treated with the only mechanical causal therapy without using chemical agents (Control sites). Sub gingival plaque, gingival bleeding (BOP+), plaque index (PI-Plaque Control Record) and Probing Depths (PD) were evaluated at baseline (prior to any treatment) and after 4 weeks (21 days after treatment).

Results: The results showed that the mechanical causal therapy has a good effect in reducing the clinical indices, but the addition of antiseptics provides a significantly improvement in the PI, BOP and frequency of periodontopathic bacteria.

Conclusion: The application of xanthan-based chlorhexidine (Xan-CHX) gel offers a great benefit in improving of the indices of periodontal disease, proving to be essential as adjunctive therapy in patients with a serious or moderate high chronic periodontitis, and of course as part of a program of periodontal treatment.

Key Words: Periodontal therapy, Chlorhexidine, Drug delivery systems, Periodontal disease, Xanthan gum.

Introduction

Periodontal disease is now widespread and occurs in people of all ages. The main causes of the evolution of this disease could be found both in the bacterial flora of the oral cavity, acting as direct damage, and in the host immune response, that provides an indirect damage, and related to a process, slow but inexorable, of aging of the periodontal structures [1-3]. This pathological phenomenon may be limited by a regular professional supervision and by a good oral hygiene. Unfortunately, the poor attention of the patient to oral diseases, the lack of information about preventive methods to be adopted (as the correct brushing techniques), the continued aging of the population, immunological defects (i.e. leukocyte abnormalities) make that the incidence of periodontitis is high and constantly increasing. From here, it's shown the importance of prevention understood as the prompt and regular removal of plaque, with periodic checks timetabled according to individual needs and targeted information and motivation to the periodontal "disease". Moreover, the immune system has a destructive potential which should not be overlooked.

This lack of prevention leads to study protocols, methods and drugs that are able to oppose to the progression of periodontal disease and they are able to help the patient in the control of plaque and therefore "help to help" to maintain the state of health of the oral cavity [4-6]. The clinician has many products, which in a more or less significant way and in combination with mechanical causal therapy, allow achieving satisfactory results [7]. As an antiseptic, chlorhexidine has been used effectively for over 30 years in the treatment of periodontal disease [8-12]. It shows a broad spectrum of topical

anti microbial activity, safety, effectiveness, substantivity and lack of toxicity. Subgingival irrigation using CHX solutions was not effective in the treatment of periodontitis because of the lack of effective concentrations within the periodontal pocket for sufficiently long times [8-12]. Slow-release CHX devices were developed to overcome this limitation. In addition to liquid and solid carrier-based devices, gel-based devices were designed. The Chlo-SITE is xanthan gum gel, which contains the 1.5% chlorhexidine, capable of ensuring, for at least two weeks, optimal conditions for active and passive disinfection, since it gives progressively high amounts of chlorhexidine into the crevicular liquid. The xanthan is a polysaccharide which with water forms a three-dimensional pseudo-plastic network, able to suspend and hold various substances, which are released gradually in relation to their physical and chemical characteristics [8].

The Chlo-Site is kept at room temperature and is supplied in disposable syringes; is applied directly with the syringe in the pocket, starting from the deepest part of the pocket to the gingival margin, thanks to the thin needle with a rounded point that does not traumatizes the tissues.

The study evaluated the effects of a xanthan-based chlorhexidine (Xan-CHX) gel used as an adjunct to Scaling and Root Planing (SRP) in patients with generalized mild-severe chronic periodontitis.

Materials and Methods

Clinical procedures and sub gingival plaque collection

Thirty non-smoking patients, 15 males and 15 females (mean

age 54.1 ± 6.9 years) were enrolled in this study. This study was conducted on patients recruited from the department of Periodontology of the Dental Clinic at the University of L'Aquila and Chieti. The subjects had to comply with the following criteria:

(i) Positive for diagnosis of mild-to-severe chronic periodontitis (generalized periodontal disease with pocket depths between 3 and 5 mm).

(ii) Good general health according to medical history, blood pressure, pulse rate and clinical judgment.

(iii) Negative for hypersensitivity to chlorhexidine.

(iv) Negative for the use of any antibiotic or anti-inflammatory drugs within the 3 months preceding the beginning of the study. Pregnant or nursing females were excluded from the study.

(v) Periodontal treatment undertaken <6 months prior to the preliminary visit.

Voluntary informed consent was obtained from the patients after providing them with detailed information about the clinical trial.

Patients were analyzed at baseline (T0, prior to any treatment), after 7 days (T1), after 4 weeks (T2). The 1st and 4th quadrant were used for the study with the application of antiseptic (Test); the 2nd and 3rd as a control; then each study group was composed of 60 test sites and 60 control sites.

At T0, a clinical monitoring was performed and at each patient the following parameters were recorded: gingival bleeding within 15 s after probing (BOP +) with a 20 g controlled-force probe, plaque index (PI-Plaque Control Record) assessed by visual criteria and Probing Depths (PD) measured as the distance from the bottom of the pocket to the most apical portion of the gingival margin. Moreover, the same operator always collected the clinical data.

During the first visit each patient was given the rules for proper use of the toothbrush and toothpaste. Each patient has repeated the indications received so as to correct any inaccuracies. Repeated Oral Hygiene Instructions (OHI), consisting of Bass' brushing technique and regarding the correct use of dental floss and an interdental brush, were given to all participants when they initially underwent SRP. The same OHI were further reinforced throughout the study. Finally, subjects were not allowed to take any antibiotics and anti-inflammatory drugs, or chlorhexidine-based mouth rinses, during the entire length of the study.

After 7 days (T1) the patient was subjected to full mouth disinfection and under local anesthesia at Scaling and Root Planing (SRP) in a single session.

At the end the pockets were dried with sterile absorbent paper cones and in two of the four quadrants (Test sites) has been applied Xan-CHX gel containing a mixture of CHX digluconate and CHX dihydrochloride (ratio 1:2) to a total of 2.5% CHX (Chlo-SITE, Ghimas, Casalecchio di Reno, BO, Italy). The gels were administered by using a syringe with a non-traumatic needle. The needle was inserted into the periodontal pocket and the gel applied around the tooth in a gentle probing manner in an attempt to include the full extent of the pocket. Gel was applied until the pocket was overfilled, leaving a residue visible at the gingival margin of all affected sites of the tooth. Care was taken to avoid any tissue injury.

At the end of the procedure in quadrants 1st and 4th has been applied antiseptic (Test sites), while the quadrants 2nd and 3rd (Control sites), were treated with the only mechanical causal therapy without the use of chemical agents.

Then, the patient was motivated again with proper oral hygiene instructions, except for the use of chemotherapeutic mouth rinses and oral irrigation devices.

At T2 (after 3 weeks) the patients were subjected to a reevaluation and BOP +, PI and PD were evaluated.

Moreover, for microbiological monitoring, sub gingival plaque was collected from two experimental pockets for quadrant at T1, prior to the SRP, and again at 21 days (T2) after the end of the sub gingival gel administration.

Sub gingival plaque was collected for microbiological evaluation as follows: the sites were first isolated with cotton rolls, and after removal of supra gingival plaque with a sterile curette (Asadental, Bozzano, Italy) the gingival surface was dried. The plaque samples were then obtained by insertion of three standardized #30 sterile paper points (Inline, Torino, Italy) into the deepest part of each periodontal pocket, and left in situ for 15 s for saturation.

The sample was then sent to the laboratory for PCR analyses.

Bacteriological methods: PCR analyses

Plaque samples were stored at 4°C in saline solution until DNA extraction (24-48 hour later). Vortexed plaque samples were centrifuged for 15min at 14000 rpm. Pellet was suspended in 300 µl lysis solution (50 mM Tris, 10 mM EDTA, 10%SDS), treated with lysozyme (5 mg/ml) and incubated for 1 hour at 37°C. Proteinase K was added and incubated for 1h at 65°C, DNA was extracted according to the method phenol/chloroform-isoamyl alcohol. Nucleic acids were precipitated in alcohol, washed with 70% (vol/vol) alcohol and suspended in bidistilled water to create the DNA template.

Each plaque was analyzed using a multiplex PCR, to reveal the following species: *Aggregatibacter actinomycetemcomitans*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Tannerella forsythia*, *Eikenella corrodens*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola* [13].

PCR was performed using ubiquitous primers with modified 16S rRNA to determine the bacterial count. Negative controls (no DNA) and Positive controls with DNA coming from pure bacterial cultures were tested. Amplification was performed in 100µl reaction, containing 10 mM Tris-HCl, pH 8.0, 50 mM KCl (1 x PCR Buffer), 1.5 mM MgCl₂, 200µM of each nucleotide, 30 pml of each primer, 2.5 U Hot Start Taq DNA Polymerase (Quiagen S.P.A. Milan Italy) and 5 µl of DNA Template. The solution was amplified in a first cycle at 98°C for 15 min to activate the polymerase, 40 cycles at the following temperatures: denaturation for 30 sec at 95°C, annealing for 1 min at 60°C, extension for 1 min at 72°C. Final extension step 10 min at 72°C. The reactions were conducted in a thermocycler iCycler System (Bio-Rad Laboratories srl, Segrate, Italy).

Amplification of ubiquitous primers was conducted in the same way. PCR products were visualized using electrophoresis on agarose gels. Experimental design included a negative control with a DNA-free template and a positive

control coming from pure cultures. PCR products were tested for specificity with restriction enzymes: Eco RI, Spe I, Xba I, Hind III, Kpm I for *C. rectus*; Dra I for *T. forsythia*; ApaI, Taq I, Sma I for *T. denticola*; Sma I for *A. actinomycetemcomitans*, *P. gingivalis*, *E. corrodens*; Dra I for *P. intermedia*.

Electrophoretic amplified products were identified by electrophoresis of 20 µl from each PCR tube, placed in agarose gel 2% buffered with TAE (Tris-Acetate-EDTA buffer) for 2 h at 80V.

Electrophoretic bands were visualized and photographed with a UV rays trans-illuminator (gel Doc 2000, Bio-Rad) after staining for 30 min with ethidium bromide (1 µg/ml). Amplified fragments were compared to a DNA marker (number VIII, Roche Diagnostics SPA, Milan Italy).

Statistical analysis

For statistical analysis the Matched-Pairs Signed-Wilcoxon-rank test have been used. Data are shown as Mean ± Standard Deviation (SD), and for all analyses a P-value of less than 0.05 was considered significant.

Results

The clinical results obtained in the test sites treated with xanthan-based chlorhexidine (Xan-CHX) gel and the results relating to the control sites, treated with only SRP, are summarized in *Table 1*.

The baseline examination revealed that both study groups demonstrated similar values for PI, BOP, and PD. In all the treated sites was obtained a reduction of the probing depth, a reduction of the bleeding in the short term and a reduction of the inflammatory state.

In particular, the results showed that the xanthan-based chlorhexidine (Xan-CHX) gel used as an adjunct to Scaling and Root Planing (SRP) led to an improvement in the values of PI and BOP statistically significant against the non-surgical periodontal treatment alone.

Table 2 shows the data relating to the assessment of oral hygiene. It is interesting to note that for all the study the subjects maintain a motivation to oral hygiene insufficient or moderate and no one achieved a discrete or good rate.

The frequencies of detection of each of the individual periodontopathic bacteria analyzed are shown in *Table 3*. At the baseline, no significant differences among the groups were noticed in the frequencies of detection of each monitored bacteria. In the non-surgical periodontal treatment group *C. rectus*, *F. nucleatum* and *T. denticola* showed significant reductions in their frequencies of detection throughout the study. In the test group (SRP + Xan-CHX) *A. actinomycetemcomitans*, *C. rectus*, *E. corrodens*, *F. nucleatum* and *T. denticola* had reduced numbers of positive sites after the treatment. When comparing the two treatment groups, the frequencies of detection of seven of the bacteria species were lower in the SRP + Xan-CHX treatment group compared to the SRP, and the difference reached statistical significance for *A. actinomycetemcomitans* and *E. corrodens*.

Discussion

The analysis of results showed that in cases of therapy supported by locally delivered antibacterials containing chlorhexidine there was observed a greater advantage, as the use of such antiseptics offers a substantial significantly

Table 1. Clinical parameters at the baseline and at the 28-day examinations in the different experimental groups.

Baseline		After 28 days		Among group differences	
Control	Test	Control	Test	Baseline	21 days after treatment
1.878 ± 0.3634	1.572 ± 0.9582	1.6667 ± 0.7581*	0.7000 ± 0.5350*	NS	P = 0.0000
0.773 ± 0.4232	0.723 ± 0.6622	0.7000 ± 0.4661*	0.3000 ± 0.4661*	NS	P = 0.0015
3.4667 ± 0.7303	3.5000 ± 0.6297	2.3000 ± 1.0222*	2.0333 ± 0.7184*	P = 0.8505 NS	P = 0.2472

- PI, BOP, PD mean values ± SD (Standard Deviation)
- PD was measured in millimeters (mm)
- * = Difference over time
- S = statistically significant
- NS = not statistically significant

Table 2. Evaluation of oral hygiene during the treatment period.

Oral Hygiene	(-) insufficient	(+) moderate	(+ +) discrete	(+ + +) good
	40	60	0	0

Data are presented as percentage of examined subjects

Table 3. Number of sites positive for the presence of each bacterial species over time in the different experimental groups.

	Baseline		After 28 days		Among group differences	
	Control	Test	Control	Test	Baseline	21 days after treatment
<i>A. actinomycetemcomitans</i>	59.2	65.3	54.3	43.2*	NS	P < 0.05
<i>C. rectus</i>	64.3	67.3	49.2*	45.3*	NS	NS
<i>E. corrodens</i>	65.3	69.4	60.3	48.7*	NS	P < 0.05
<i>F. nucleatum</i>	72.4	62.2	57.7*	48.8*	NS	NS
<i>P. gingivalis</i>	44.9	40.8	35.2	33.7	NS	NS
<i>P. intermedia</i>	37.8	34.0	30.7	25.6	NS	NS
<i>T. forsythia</i>	37.8	38.8	35.4	30	NS	NS
<i>T. denticola</i>	45.9	52.6	32.5*	40.2*	NS	NS

- Data are presented as percentage of positive sites
- NS = No statistically significant difference
- * = statistically significant difference from the corresponding baseline score

improvement in the plaque index with reduction of the periodontopathic bacteria and consequent improvement in bleeding index as regards the non-surgical therapy alone.

In both types of treatment there was obtained a reduction of inflammatory diseases in agreement with studies by numerous authors [8,10-12,14-18].

The causal therapy has no mechanical limits in the clinical application if not related to the individual tolerance of the patient to such treatment (operative time, awareness, trauma of teeth, possible pain symptoms) [10,16].

On the contrary the use of antiseptics may present limitations related to the same pharmacological principle with possible sensitization towards the products [14-18].

However, these substrates offer valuable help especially where there are poorly cooperative patients or because of a lack of dexterity they cannot achieve an adequate plaque control [19-21].

The presence of test and control sites within the same mouth avoided all the variables related to individual susceptibility of each individual patient to treatment, whether pharmacologic or mechanical.

From the results obtained it was possible to note how the use of Chlo-SITE improved the disease in patients with generalized mild-severe chronic periodontitis. Although this study has been carried out for a short period, on a restricted number of patients, we can see the effectiveness of the Chlo-SITE, both in the improvement of the clinical and microbiological indices. This outcome probably would not be achieved without the aid of a good oral hygiene and the use of a product with controlled release of chlorhexidine. Patients also reported in this context, as in the daily oral hygiene procedures, they had not more gingival bleeding and

halitosis problems. Also the subjects treated with Chlo-SITE showed soft tissues healthy, pink, devoid of the typical signs of inflammation. The improvement of the periodontal tissues depend largely on the reduction of pathogen loads, the Chlo-SITE, in fact, has an antimicrobial activity against periodontal pathogens such as to alter the biochemical function and metabolism, thus reducing virulence. These results were obtained both thanks to a good compliance by patients, that the application of the Chlo-SITE that interfere with the recolonization of the microorganisms in the pocket for at least three weeks.

Supportive therapy with antiseptics may be used in combination with mechanical therapy in sites that do not respond to mechanical therapy alone, offering a valuable aid in the control of infection and also in the control of bacterial plaque, therefore useful in preventing possible recurrence of such processes inflammatory [22]. The use of antiseptics should be avoided in individuals with a hypersensitivity to the drug and even though it should be emphasized substantially topical and non-systemic use of these products [23,24].

The results of this study underline that the application of xanthan-based chlorhexidine (Xan-CHX) gel offers a great benefit in improving of the indices of periodontal disease, proving to be essential as adjunctive therapy in patients with a serious or moderate high chronic periodontitis, and of course as part of a program of periodontal treatment. In conclusion is necessary to emphasize the importance of the collaboration of the patient in such a way that it is the "key" of the success of any periodontal therapy since only a constant and significant oral control of the bacterial load during the treatment of oral hygiene methods, it allows to obtain optimal clinical outcomes in the long term.

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