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Adjunctive effect of chlorhexidine antiseptics in mechanical periodontal treatment: first results of a preliminary case series

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Abstract: *Objectives:* The aim of the present case series was to evaluate the clinical and microbiological effects of a single session of mechanical and manual scaling and root planing (SRP) combined with the use of two different chlorhexidine formulations in the treatment for generalized chronic periodontitis. *Methods:* Ten patients affected by chronic periodontal disease with periodontal probing depth (PPD) $\geq$ 5 mm were treated with SRP plus local chlorhexidine. In each patient, similar teeth, treated with SRP with the adjunctive use of chlorhexidine digluconate and dihydrochloride or chlorhexidine gluconate, respectively, were selected and assigned to a test and a control group. In both groups, PPD, bleeding on probing (BOP) parameters, total bacterial counts (TBC) and quality of periodontal bacteria at time 0 and 6 weeks after treatment were measured. *Results:* PPD significantly decreased over time both in the test and in the control group; however, no significant differences between the two groups were observed. BOP and TBC were significantly lower in the test than in the control group 6 weeks after treatment. In the post-treatment revaluation, a significant decrease both in the treatment and in the control group, for each of the single periodontal pathogens, was observed. *Conclusion:* In this study – a preliminary case series with small sample size and short follow-up – the adjunctive use of chlorhexidine (CHX) to SRP resulted in clinical and microbiological benefits in the treatment for generalized chronic periodontitis. A CHX gel formulation consisting of CHX digluconate and CHX dihydrochloride seems to lead some additional benefits over SRP plus CHX gluconate in the short term. Additional investigations are needed to evaluate the effectiveness of this antiseptic therapy.

Key words: chlorhexidine; instrumentation; micro-organism; periodontitis

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Introduction

Periodontal diseases are caused by mixed infections with the subgingival microbiota. While hundreds of different bacterial species can colonize the oral cavity, it is generally accepted that specific microorganisms are found more frequently in periodontal lesions (1, 2). Many bacterial species can be implicated in the aetiology and pathogenesis of periodontitis. Haffajee and Socransky (3) suggested the following pathogens: *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus* (*Tannerella forsythia*) and *Treponema denticola*. Some other species can play a role in the developing of periodontal disease, and probably all

pathogens have not yet been identified. The groups of bacteria above-mentioned have been detected in large numbers in the subgingival biofilm of periodontal pockets (4, 5). The virulent of periodontal pathogens, the local environment and the host susceptibility are probably the main factors involved in the progression of periodontal disease (6). Numerous investigations showed that the main therapeutic approach for periodontal disease is mechanical treatment of root surfaces via scaling and root planing (SRP) (7–10). Mechanical instrumentation is an effective method for removing supra- and subgingival bacterial biofilm adherent to tooth surfaces as well as reducing probing depths, and it produces marked changes in the subgingival bacteria to maintain a microflora compatible with periodontal health at the completion of the active phase of treatment (11). Unfortunately, SRP does not necessarily eliminate all of the periodontal pathogens. According to Petersilka (12), up to 30% of the total surface area of treated roots can be covered with residual calculus following subgingival scaling and the bacterial re-colonization of the root surface by pathogenic bacteria may lead to periodontal disease recurrence. In this respect, combined treatment of mechanical instrumentation associated with local application of antiseptics agents has been widely investigated. Conflicting results were reported regarding the advantages of the adjunctive use of antimicrobial agents – antiseptics/antibiotics – to mechanical plaque control alone. The additional use of an antimicrobial agent to mechanical plaque control could enhance the effect of therapy and result in a retarded subgingival recolonization. Hallmon (13), in a systematic review, found limited advantages in the addition of local anti-infective therapy to mechanical periodontal debridement. Hanes (14), in a systematic review, referred that in some populations, anti-infective agents in a sustained-release vehicle alone can reduce probing depth and bleeding on probing (BOP) equivalent to that achieved by SRP alone, but no evidence was found for an adjunctive effect on reduction in pocket depths (PD) and BOP of therapist-delivered CHX irrigation during SRP compared to SRP alone. Cosyn (15), in a systematic review, reported that the additional value of the chlorhexidine chip when used as an adjunct to SRP is limited and conflicting. Also Puig Silla (16), in a review of the literature, showed the need for new studies to assess the effects over the long term of chlorhexidine in patients with chronic gingivitis and periodontitis. Nevertheless, many antiseptics including chlorhexidine, hydrogen peroxide or antibiotics such as tetracycline, penicillins, metronidazole were more extensively investigated for systemic or local use in periodontal therapy or in supportive periodontal therapy tough with doubtful results. Chlorhexidine is certainly the most widely studied antiseptic with excellent plaque inhibitory properties (17, 18). Chlorhexidine is available in three forms: the digluconate, acetate and hydrochloride salts. Most oral formulations have used the digluconate salt that is water-soluble, while hydrochloride is very sparingly soluble in water.

In oral use, CHX has been reported to have a number of local side effects that are mainly reversible cosmetic problems as brown discoloration of the teeth and the dorsum of the tongue or taste perturbation. In rare cases, CHX can give more serious adverse effects as oral mucosal erosion, parotid swelling, enhanced supragingival calculus formation (19).

The substantivity of a drug in the periodontal pocket is an important factor determining its effect on the subgingival flora. Several attempts have been made to develop local delivery devices for the subgingival application of antiseptic, rather than antibiotic agents. Acrylic strips, ethyl-cellulose compounds, custom-fabricated trays, bioabsorbable chips have been tested for this purpose. CHX when used as an irrigant or vehicled in gels has the important limitation of its high clearance from the pocket due to the cleansing action of the crevicular fluid. Subgingival irrigations were shown not to be effective because of the lack of significant concentrations for sufficient lengths of time within the periodontal pockets (20). To overcome this limitation, 'slow-release devices' have been developed, including 'sustained-release devices' that deliver CHX for <24 h and 'controlled-delivery devices' that release CHX over an extended period of time (21). Therefore, one of the predominant factors in the development of a sustained-release delivery device is the ability to control the rate of release of the drug over time. Any antiseptic formulation aiming to provide a sustained effect in the subgingival environment must include a vehicle with intrinsic capacity to maintain antimicrobial levels beyond concentration breakpoints during sufficient time. To date, most authors agree that additional randomized clinical trials (RCTs) are needed, which evaluate the effectiveness of antiseptic therapies in all forms of periodontitis.

The aim of this study was to evaluate the clinical and microbiological effects of two local different chlorhexidine formulations – chlorhexidine digluconate and dihydrochloride versus chlorhexidine gluconate – in addition with SRP for the treatment of selected periodontal pockets of patients affected by generalized chronic periodontitis.

Materials and methods

Ten systemically healthy patients, aged 38–45 years, were recruited from new referrals to Department of Oral Hygiene, Dental Clinic of University Vita-Salute San Raffaele, Milan. Inclusion criteria were (i) clinical diagnosis of generalized chronic periodontitis; (ii) two non-adjacent sites per quadrant with PD of 5mm or over and radiographic evidence of bone loss, with no history of systemic disease or antiseptic/antibiotic therapy within the last 3 months or during the course of the study; (iii) the willing to provide informed consent and to ensure compliance throughout the study. Exclusion criteria were (i) pregnancy or lactation; (ii) adverse effects to CHX; (iii) systemic and/or topical antibiotics during the last 3 months; (iv) cigarette smoking; (v) physical or mental handicap. All patients gave informed consent. The study design was approved by the local Ethical Committee and was found to conform the requirements of the Declaration of Helsinky.

In this study, we used a *Test formulation* that was a xanthan gel of chlorhexidine digluconate 0.5% and chlorhexidine dihy-

drochloride 1% (Chlo Site; Ghimas SpA, Bologna, Italy) and a *Control formulation* that was a gel of chlorhexidine gluconate 1% (Corsodyl gel; GlaxoSmithKline SpA, Milano, Italy). The two formulations were provided in identical packing so that neither the patient nor the investigator was aware of which treatment had been assigned (double-blinding, allocation concealment). The oral cavity of each patient was randomly divided into two equivalent parts (split-mouth study). At time 0 (baseline), the half-dental arch of the patients was assigned to either the test or the control treatment according to a randomization list and two non-adjacent teeth according to the inclusion criteria were selected. The *Test group* was formed by two upper incisors, two upper canines, two upper premolars, three upper molars and one lower molar. The *Control group* was formed by one upper incisor, two upper canines, two upper premolars, three upper molars and two lower molars. At baseline, periodontal parameters – periodontal probing depth (PPD), BOP – were recorded, and plaque samples were collected. At week 1, The *Test group* received a single session of full-mouth SRP associated with subgingival administration of a single-dose syringe with 0.25 mg of the CHX Test formulation; the *Control group* received identical mechanical treatment associated with subgingival administration of a single-dose syringe with 0.25 mg of CHX Control formulation. SRP was performed by the same operator by means of ultrasonic device and specific tips (C1 EMS Italia S.p.a) and by hand mechanical instrumentation by means of standardized Gracey curettes (Hu-Friedy, Chicago, IL, USA). Mechanical debridement was supplemented by supragingival polishing with a non-fluoridated prophylaxis paste. Working time during the instrumentation session was standardized (2 h) (*22*). Personalized oral hygiene instructions were verbally provided.

Clinical recordings

At week 0 and 6, PPD and BOP were recorded at four sites (mesio-buccal, disto-buccal, mesio-lingual, disto-lingual) on the selected teeth. PPD was measured by means of a standard periodontal probe (PCPUNC15; Hu-Friedy) with a manual pressure of approximately 25 g; BOP was recorded with the same instrument and was considered positive if bleeding occurred between 30 seconds after probing.

Microbiological samples

At week 0 and 6, subgingival plaque samples were collected at four sites for each tooth according to a standardized procedure. The sites were isolated with cotton rolls. After removal of the supragingival plaque using a sterile Gracey curette, a standardized sterile paper point was inserted into the deepest part of each periodontal pocket, which was left *in situ* for 20 s. The paper points were then transferred to a test tube containing a transport medium and sent to the microbiological laboratory (IAI Institut, Zuchwil, Switzerland). Polymerase Chain Reaction method was used for detection and qualification of total bacterial counts (TBC) as well as periodontal bacteria as *Actinobacillus actinomycetemcomitans* (Aa), *Tannerella forsythia* (Tf), *Porphyromonas gingivalis* (Pg), *Treponema denticola* (Td) (Gum PerioCheck Sunstar Srl, Varese, Italy).

Data analysis

Data from clinical and microbiological parameters were analysed using statistical software (SPSS; SPSS Inc., Chicago, IL, USA). Data were expressed as the median (Mdn) and interquartile range (IR). Pairwise comparisons were made to detect significant intra- and inter-treatment differences (Wilcoxon non-parametric signed ranks test for related observations). The decision criterion for statistical significance was set at $\alpha = 0.05$ (i.e. $P < 0.05$ for hypothesis testing).

Results

Descriptive statistics for PPD are summarized in Table 1. Baseline PPD median values (Mdn) were equivalent both in the test and in the control group ($P = 0.526 > 0.05$). Further, PPD significantly decreased over time both in the test ($P = 0.005$) and in the control group ($P = 0.005$). No significant difference in PPD was observed between test and control group 6 weeks after treatment ($P = 0.441 > 0.05$).

Descriptive statistics for BOP are summarized in Table 2. Baseline BOP median values (Mdn) were equivalent both in the test and control group ($P = 0.127 > 0.05$). Further, BOP significantly decreased over time both in the test ($P = 0.007$) and in the control group ($P = 0.007$). A significant difference between test and control group was observed 6 weeks after treatment ($P = 0.042$). At closer inspection, also the values defining the lower and upper limits of the inter-quartile range (IR) observed in the test group 6 weeks after treatment (IR = 0–16.67) resulted ostensibly smaller than those observed at baseline both in the control (IR = 62.50–100) and in the test groups (IR = 50.00–75.00). This reduced range of observed values in the treatment group 6 weeks after the intervention implies that the treatment tended to produce *homogenously* positive, clinical outcomes on BOP.

Descriptive statistics for TBC are summarized in Table 3. Baseline TBC median values (Mdn) were equivalent both in

Table 1. **Probing pocket depth as assessed in 10 selected teeth in Test and Control group**

	Control group PPD		Test group PPD		
	Mdn	IR	Mdn	IR	*P*-value*
T_0 baseline	5.88	5.00–6.13	5.78	5.25–6.44	0.526
T_1 6 weeks	4.13	3.50–4.50	3.74	3.19–4.19	0.441
P-value**	0.005		0.005		

**P*-value for pairwise inter-group comparisons (Wilcoxon signed ranks test for related samples).
***P*-value for pairwise intra-group comparisons (Wilcoxon signed ranks test for related samples).
IR, interquartile range; Mdn, median; PPD, probing pocket depth.

Table 2. **Bleeding on probing as assessed in 10 selected teeth in Test and Control group**

	Control group BOP		Test group BOP		
	Mdn	IR	Mdn	IR	*P*-value*
T_0 baseline	83.33	62.50–100	66.67	50–75.00	0.127
T_1 6 weeks	16.67	12.50–33.33	8.34	0–16.67	0.042
P-value**	0.007		0.007		

**P*-value for pairwise inter-group comparisons (Wilcoxon signed ranks test for related samples).
***P*-value for pairwise intra-group comparisons (Wilcoxon signed ranks test for related samples).
BOP, bleeding on probing; IR, interquartile range; Mdn, median.

Table 3. **Total bacterial count as assessed in 10 selected teeth in Test and Control group**

	Control group TBC		Test group TBC		
	Mdn	IR	Mdn	IR	*P*-value*
T_0 baseline	46.74	31.41 – 80.75	49.50	44.08–69.96	0.889
T_1 6 weeks	25.64	8.29 – 42.78	11.14	7.00 – 18.00	0.037
P-value**	0.074		0.005		

**P*-value for pairwise inter-group comparisons (Wilcoxon signed ranks test for related samples).
***P*-value for pairwise intra-group comparisons (Wilcoxon signed ranks test for related samples).
IR, interquartile range; Mdn, median; TBC, total bacterial count.

the test and in the control group ($P = 0.889 > 0.05$). Further, TBC significantly decreased over time in the test ($P = 0.005$) but not in the control group ($P = 0.074 > 0.05$). A significant difference between test and control group emerged 6 weeks after treatment ($P = 0.037$). Again, at closer inspection of Table 3, also the values defining the lower and upper limits of the inter-quartile range (IR) observed in the test group 6 weeks after treatment (IR = 7.00–18.00) resulted ostensibly smaller than those observed at baseline both in the control (IR = 31.41–80.75) and in the test groups (IR = 44.08–69.96). This reduced range of observed values in the treatment group (6 weeks after the intervention) shows that the treatment tended to produce *homogenously* positive outcomes also on TBC.

Table 4 summarizes statistical data for periodontal pathogens. Aa was detected in one single patient – this preventing any statistical analysis for this kind of bacteria. For the remaining species – Tf, Pg, Td – median values at baseline were equivalent both in the test and in the control groups (Tf: $P = 0.141$; Pg: $P = 0.786$; Td: $P = 0.686$). In the post-treatment revaluation (6 weeks after treatment), a significant decrease, relative to baseline, was observed for all microbial species in both groups (Tf: Test $P = 0.018$, Control $P = 0.017$; Pg: Test $P = 0.018$, Control $P = 0.027$; Td: Test $P = 0.043$, Control $P = 0.042$). As 6 weeks after treatment, the count of periodontal pathogens reached zero both in the control and in the treatment group, no statistically significant difference emerged between the two groups.

Table 4. **Periodontopathic bacteria – *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola* – as assessed in 10 selected teeth in Test and Control group**

	Control group Tf		Test group Tf		
	Mdn	IR	Mdn	IR	*P*-value*
T_0 baseline	0.65	0,00–1 36	1.00	0.00 – 2.21	0.141
T_1 6 weeks	0.00	0,00–0 00	0 00	0.00 – 0.00	0.317
P-value**	0.017		0.018		
	Pg		Pg		
	Mdn	IR	Mdn	IR	
T_0 baseline	0.47	0.00–4.00	2.67	0.00–4.09	0.786
T_1 6 weeks	0.00	0.00–0.00	0.00	0.00 – 0.00	0.317
P-value**	0.027	0.018			
	Td		Td		
	Mdn	IR	Mdn	IR	
T_0 baseline	0.05	0.00 – 1.00	0.01	0.00 – 0.81	0.686
T_1 6 weeks	0.00	0.00 – 0.00	0.00	0.00 – 0.00	1.000
P-value[(2)]	0.042	0.043			

**P*-value for pairwise inter-group comparisons (Wilcoxon signed ranks test for related samples).
***P*-value for pairwise intra-group comparisons (Wilcoxon signed ranks test for related samples).
IR, interquartile range; Mdn, median; Pg, *Porphyromonas gingivalis*; Td, *Treponema denticola*; Tf, *Tannerella forsythia*.

Discussion

The present clinical and microbiological study evaluated the effects of a single session of mechanical and manual SRP combined with the professional use of two different CHX formulations in the treatment for generalized chronic periodontitis. The clinical and microbiological parameters assessed at the baseline observation were revaluated after 6 weeks. The aim of this study was to provide further data on the effects of different formulations of CHX when used as an adjunct to SRP.

Marked improvements in all clinical indices were detected after both treatment modalities with some differences between test and control groups. This study showed the same magnitude of PPD reduction in deep pockets between the two treatment modalities, while the BOP decrease was notably greater for the test group where SRP was performed in adjunction with chlorhexidine digluconate and dihydrochloride. PPD and BOP significant reductions from baseline values do completely agree with precedent findings where SRP with or without the adjunctive use of antimicrobials improves over time the probing depth and the BOP of periodontal pockets (23–25). The mechanical removal of supra- and sub-gingival plaque leads to a marked reduction in periodontal tissue inflammation and the use of antimicrobial agents might result in a delayed recolonization of dental roots. TBC was significantly lower only in the test group at the final observation. This result disagrees with previous clinical trials attesting that post-SRP treatment, the

majority of the organisms are detected in significantly lower frequencies than baseline (23, 26, 27). Our TBC finding is difficult to explain. Both treatment modalities resulted in marked reductions in the percentage of periodontal pathogens. For most of the bacteria tested following both treatments, the majority of the microorganisms were not still detected post-mechanical plus antiseptic strategy. This is in contrast with other reports which showed that SRP with or without antimicrobial treatment lowers the numbers of colonies of periodontal pathogens, but is unlikely to eliminate completely this species (28–30). In considering this unexpected finding, we should not forget, however, that it could also stem from a (too) small sample size – which, in turn, leads to reduced statistical power (31).

The evaluation of the therapeutic efficacy of an antiseptic subgingival administration in association with mechanical periodontal treatment is widely ambiguous due to differences among trials with respect to the type of antiseptic used, the dose/frequency of administration, the therapeutic regimen. Previous studies where CHX was used in association with SRP showed no difference in clinical and microbiological parameters compared to SRP treatment alone. On the contrary, Cosyn (32), in a controlled trial, reported microbiological results promoting the subgingival administration of a highly concentrated CHX varnish as an adjunct to same-day full-mouth root planing. Also, Paolantonio (22) showed that the adjunctive use of CHX chip with SRP resulted in a clinically meaningful improvement in PPD reduction compared to SRP alone, but no differences were found in BOP, TBC and frequencies of detection of each of the periodontal pathogens. More recently, Gonzales (33), in a randomized trial, reported that the use of CHX chips before and immediately after SRP improves clinical attachment loss and reduces the subgingival microorganisms of the red complex in the treatment for chronic periodontitis.

Moreover, previous findings demonstrated that the adjunctive daily use of an antimicrobial agent to mechanical plaque control may result in a retarded subgingival recolonization by periodontal pathogens in patients affected by generalized chronic or aggressive periodontitis (34–36). CHX is to date the proven most effective plaque inhibitory agent for both preventive and therapeutic treatment with local side effects that are mainly cosmetic problems. Mouthrinses, gel, sprays, varnishes of CHX digluconate have been used extensively for anti-plaque properties. In our study, we matched two different gel CHX formulations – chlorhexidine digluconate and dihydrochloride versus chlorhexidine gluconate. The peculiarity of the first formulation is the presence of a water-soluble salt (CHX digluconate) together with a rather water-soluble component (CHX hydrochloride). The CHX formulation tested is also manufactured as a xanthan gel. Xanthan is a saccharide polymer that together with water forms a three-dimensional plastic network able to retain various substances that are released gradually. The rationale for the adjunctive use of xanthan gum in a subgingival gel carrier relates to the increased viscosity of the carrier and in the bioadhesive properties of the polysaccharides, both of which may limit the clearance of CHX from the periodontal pocket. A xanthan gel chemically linked to the CHX molecule has demonstrated *in vitro* its capacity to maintain adequate CHX concentrations and a highly stable pharmacokinetic profile inside the periodontal pocket. In this formulation, the release of CHX digluconate lasts 6–10 days, while CHX dihydrochloride is released in the following days and maintains the bacteriostatic and bactericidal concentrations for at least 2 weeks, according to the manufacturer's opinion (37, 38).

Paolantonio (39), in a randomized multicentre trial, reported that the adjunctive use of xanthan–CHX gel with SRP resulted in a clinically significant improvement in PPD reduction and CAL gain compared to SRP alone. These results were concomitant with the significantly greater effects that xanthan–CHX gel treatment exerted on the subgingival microbiota.

Matesanz (38), in a randomized clinical trial, showed that SRP with adjunctive subgingival application of a xanthan-based CHX gel may improve, although to a limited extent, the clinical outcomes in chronic periodontitis patients with 'residual' or 'relapsing' pockets.

In our study, the main differences between the two CHX formulations tested were the BOP and TBC decrease after 6 weeks from the baseline. No differences were seen in the other clinical and microbiological parameters. However, due to the limited sample size of the study population and the short-term observation, further controlled trials need to be conducted to confirm these preliminary findings also because some results are not consistent with previous studies. In conclusion, the adjunctive use of CHX to SRP in the treatment for periodontal disease is to date a doubtful concern. With the limit of this study – a preliminary case series with small sample size and short follow-up – the adjunctive use of CHX to SRP resulted in clinical and microbiological benefits in the treatment for generalized chronic periodontitis. A CHX gel formulation consisting of chlorhexidine digluconate and chlorhexidine dihydrochloride seems to lead some additional benefits over SRP plus chlorhexidine gluconate in the short term. Our preliminary results are worthy of further investigations to evaluate the effectiveness of this antiseptic therapy. Right now no definitive conclusion can be stated.

Conflicts of interest

This is to state that there is no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations that could have inappropriately influenced the results of the study.

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