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Clinical and microbiological effects of subgingival administration of two active gels on persistent pockets of chronic periodontitis patients

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Abstract

Objectives: The present controlled, single-blind study was performed to assess and compare the clinical healing and the microbiological findings following repeated intrasulcular applications of 1% metronidazole or 1% chlorhexidine gels in persistent periodontal pockets previously treated by scaling and root planing (SRP).

Material and Methods: Sixty-three systemically healthy subjects, 25 males and 38 females (mean age 48.4 ± 7.2 years), diagnosed for chronic periodontitis were enrolled in this study. They underwent SRP and received oral hygiene instructions (OHI). Three months later, at baseline, a single persistent pocket with a probing depth (PD) of 5–9 mm was chosen as the experimental site in each patient; the subjects were stratified into three matched experimental groups on the basis of the treatment to be performed, which consisted of the subgingival administration of 1% metronidazole gel (MG, $n = 19$), 1% chlorhexidine gel (CG, $n = 20$) or placebo gel (PG, $n = 24$). The treatments consisted of four repeated administrations of subgingival gels, each separated by 7 days, starting at the baseline. Clinical assessment was performed at the baseline and at the 180-day follow-up, after the end of treatment. For microbiological evaluations, subgingival plaque was sampled from the experimental sites at baseline, prior to the first subgingival gel administration, and at 7, 15, 30 and 90 days after the end of the treatment (days 28, 36, 51 and 111 from baseline).

Results: Plaque accumulation did not change significantly in all three groups. Bleeding on probing and clinical attachment levels reduced in the MGs and CGs only. PD was significantly reduced by the same amount in all experimental groups. In the MGs and CGs a remarkable reduction in the frequencies of detection of several periodontopathic micoorganisms was recorded after the treatment. The same was not seen for the PGs.

Conclusions: Subgingival administration of MG or CG, both at 1%, may have a role in the management of persistent pockets during chronic periodontitis.

Key words: chlorhexidine; inflammation; metronidazole; periodontitis; residual pockets; subgingival gel

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It is well established that periodontal disease is the result of a local bacterial infection by a pathogenic microflora within the periodontal pocket (for a review, see Offenbacher 1996). Moreover, it has been shown that only pockets of less than 3 mm in depth can be maintained plaque-free by home care and that regular professional non-surgical care can maintain the stability of deeper pockets over the years (Berkey et al. 1995, Kaldahl et al. 1996).

Currently, the most common therapy for periodontal inflammatory diseases consists of repeated professional

supra- and subgingival plaque and calculus removal (scaling and root planing, SRP) (Badersten et al. 1981). However, after non-surgical therapy, several deep periodontal pockets may persist, and in such a case the treatment consists of surgical procedures (Barrington 1981). Moreover, antibiotics and antiseptics have been successfully used to treat moderate-to-severe periodontal disease (Rams & Slots 1996), both with systemic (Noyan et al. 1997, Palmer et al. 1998) and local administration (Unsal et al. 1994, 1995, Lie et al. 1998, Knoll-Kohler 1999, Griffiths et al. 2000, Vinholis et al. 2001).

It appears that the local application of antimicrobials that are effective against periodontopathogens can reduce periodontal pocket depths (Okuda et al. 1992). Controlled-release antimicrobial delivery systems have also been tested as monotherapies, independent of SRP (Pedrazzoli et al. 1992, Unsal et al. 1995, Stelzel & Flores-de-Jacoby 1997, Awartani & Zulqarnain 1998, Rudhart et al. 1998, Knoll-Kohler 1999) or in combination with mechanical debridement as adjunctive therapies (Unsal et al. 1994, Vandekerckhove et al. 1996, Noyan et al. 1997, Awartani & Zulqarnain 1998, Lie et al. 1998, Palmer et al. 1998, Kinane & Radwar 1999, Riep et al. 1999, Griffiths et al. 2000, Stelzel & Flores-de-Jacoby 2000, Vinholis et al. 2001).

As an antiseptic, chlorhexidine has been used effectively for over 30 years in the treatment of periodontal disease (Hugo & Longworth 1964, 1965). It shows a broad spectrum of topical antimicrobial activity, safety, effectiveness, substantivity and lack of toxicity (Löe & Schiott 1970). However, as a frequent undesirable side-effect, the pigmentation of teeth and oral tissues may occur after prolonged use (Eriksen et al. 1985).

Several studies have focused their attention on the use of metronidazole for the treatment of periodontal disease. This drug is accumulated by obligate anaerobic bacteria, and leads to cell death by interfering with the synthesis of nucleic acids. Some studies have tested the efficacy of systemic metronidazole during periodontal disease (Noyan et al. 1997, Palmer et al. 1998), while others have tested the topical application of metronidazole directly into the infected pocket either alone (Pedrazzoli et al. 1992, Noyan et al. 1997, Stelzel & Flores-de-Jacoby 1997, Knoll-Kohler 1999) or as an adjunct to mechanical debridement (Awartani et al. 1998, Kinane & Radwar 1999, Riep et al. 1999).

However, to date, only one study (Salvi et al. 2002) has evaluated the effectiveness of PerioChip® (Perio Products, Jerusalem, Israel), Elyzol® Dental Gel (Dumex, Copenhagen, Denmark) and Atridox™ (Block Drug Corporation, Jersey City, NJ, USA) in treating residual periodontal pockets of chronic periodontitis patients under supportive periodontal treatment. This clinical trial did not include any control or placebo group. Moreover, the effects of repeated intrasulcular administration of 1% metronidazole gel (MG) or 1% chlorhexidine gel (CG) in residual pockets of maintenance patients still remains to be tested. Therefore, the aim of the present placebo-controlled clinical trial was to assess and compare the clinical healing and the microbiological findings following such treatments, in order to assess whether they can be used after the SRP phase of periodontal treatment as an adjunct in the treatment of persistent periodontal pockets.

Materials and Methods

Study population and design

Sixty-three non-smoking patients, 25 males and 38 females (mean age 48.4 ± 7.2 years; aged between 39 and 58 years) were enrolled in this study. The subjects had to comply with the following criteria: (i) positive for diagnosis of mild-to-severe chronic periodontitis, (ii) good general health according to medical history, blood pressure, pulse rate and clinical judgment, (iii) negative for hypersensitivity to metronidazole and/or chlorhexidine, (iv) negative for the use of any antibiotic or anti-inflammatory drugs within the 6 months preceding the beginning of the study. Pregnant or nursing females were excluded from the study. Each subject was treated by SRP under local anaesthesia in four clinical sessions at 1-week intervals by the same operator and no further mechanical instrumentation was performed during the following 3 months. At this 3-month follow-up, considered as the baseline (see Fig. 1), all the patients presented a $\leqslant 20\%$ full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS), obtained as discussed below. One persistent pocket of 5–9 mm probing depth (PD) surrounding a molar/premolar with a clinically healthy crown (which was not a third molar or a severely malpositioned tooth) was chosen as the experimental site in each patient. Moreover, an attempt was made to select a comparable number of sites, from the upper and lower dental arches, among the three groups. At baseline, the subjects were stratified into three matched experimental groups. In two test groups, the persistent periodontal pockets of the patients were treated by means of one of two active subgingival gels, containing either 1% metronidazole (Metronidazolo SAME, SmithKline Beecham spa, Milano, Italy) (MGs, $n = 19$) or 1% chlorhexidine (Corsodyl Dental Gel, SmithKline

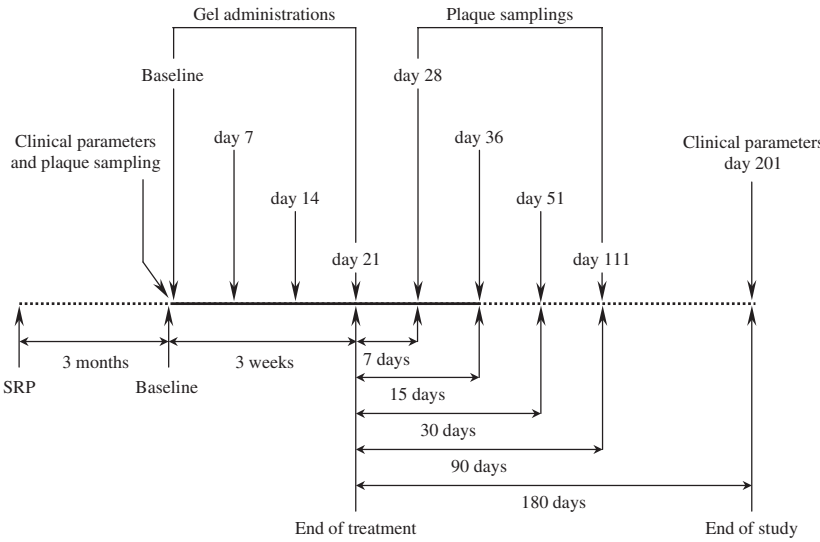

Fig. 1. Diagram of the study design.

Beecham, München, Germany) (CGs, $n = 20$). In the third, placebo, group (PGs, $n = 24$), the persistent periodontal pockets were treated with subgingival gel containing glycerine (placebo). In each of the three groups, the chemotherapeutic treatment consisted of four professional administrations of the corresponding subgingival gel, each at 7-day intervals starting from the baseline (see Fig. 1). Clinical monitoring, as reported below, was performed at the baseline and 180 days after the end of subgingival gel administration (201 days from the baseline, see Fig. 1). Moreover, for microbiological monitoring, subgingival plaque was collected from each experimental pocket at the baseline, prior to the gel administration, and again at 7, 15, 30 and 90 days after the end of the subgingival gel administration (28, 36, 51 and 111 days from the baseline, see Fig. 1). 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. Informed consent was obtained from the patients prior to the commencement of the study, and the protocol was reviewed and approved by the Ethical Committee of the G. D'Annunzio University Medical Faculty.

Clinical procedures and subgingival plaque collection

FMPS and FMBS were recorded as the percentage of tooth surfaces (mesio, distal, buccal, palatal/lingual surfaces in each tooth) with the presence of supragingival plaque or bleeding within 15 s after probing with a 20 g controlled-force probe (Vivacare TPS Probe, Vivadent, Schaän, Lichtenstein). Moreover, with each experimental site, the clinical examinations consisted of recording the presence of: (i) supragingival plaque (PL+), assessed by visual criteria, (ii) gingival bleeding within 15 s after probing (BOP+) with a 20 g controlled-force probe, (iii) PD and (iv) clinical attachment level (CAL). In particular, PD and CAL were measured as the distance from the bottom of the pocket to the most apical portion of the gingival margin and the cemento-enamel junction, respectively. Moreover, the same operator (CC) always collected the clinical data.

The gels were administrated 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.

Subgingival plaque was collected for microbiological evaluation as follows: the sites were first isolated with cotton rolls, and after removal of supragingival plaque with a sterile curette (Asadental, Bozzano, Italy) the gingival surface was dried with a gentle, sterile oxygen-free CO_2 gas flow; the plaque samples were then obtained by insertion of three standardised #30 sterile paper points (Inline, Torino, Italy) into the deepest part of each periodontal pocket, and left in situ for 15 s for saturation.

Bacteriological methods

Paper points from each patient were pooled in 2 ml of 0.1 M phosphate buffer, maintained at a temperature of $+8^{\circ}C$ ($\pm 1^{\circ}C$) in a portable electric refrigerator (International PBI SpA, Milano, Italy) and processed within 60 min from sampling. Patient samples were dispersed by vortexing for 30 s, and each sample was subjected to a series of 10-fold dilutions (to 10^{-4}) in 0.1 M phosphate buffer. Aliquots of 100 µl from each dilution were spread onto Columbia Blood Agar (CBA) plates (Oxoid Italia SpA, Garbagante Milanese, Milano, Italy) for total anaerobic viable count. Generally, isolation of microorganisms (listed in Table 2) was carried out by methods previously reported (Finegolds & George 1989, Drasar & Roberts 1991, Levett 1991). In particular, for some bacterial strains, special microbiological procedures were applied. In brief, Trypticase Soy horse serum bacitracin (75 mg/l)/Vancomycin (5 mg/l) (TSVB) plates (Slots 1982) were inoculated for assessment of *Actinobacillus actinomycetemcomitans* and incubated for 3 days in a microaerophilic environment (95/5, N_2/CO_2). Furthermore, the following were inoculated and incubated a 37°C for 7 days in an anaerobic chamber (80/10/10, $N_2/H_2/CO_2$; Don Whitley Scientific Ltd; International PBI SpA, West Yorkshire, UK): trypticase soy crystal violet erythromycin (4 mg/l) (CVE) plates (Walker et al. 1979) to assess *Fusobacterium* spp.; brucella agar (BA) plates enriched with 5% defibrinated horse blood, 0.5% hemolyzed blood and 5 mg/l menadione to assess *Peptostreptococcus micros* and the black-pigmented *Porphyromonas gingivalis* and *Prevotella intermedia* (Dahlen et al. 1993); veillonella agar vancomycin (1 mg/ml) (VAV) plates for assessment of *Veillonella parvula* (Drasar & Roberts 1991). The purification and characterization of all representative isolates (Table 2) was carried out essentially as described previously (Summanen 1992, Dahlen et al. 1993, Piccolomini et al. 1998).

Data analysis

The Statistical Package for Social Sciences software (SPSS version 8.0, SPSS® Inc., Chicago, IL, USA) was used to perform the data analysis. Parametric analyses were performed following the required assumptions verified. The balancing of experimental groups by age and sex was tested by a one-way analysis of variance (one-way ANOVA) and χ^2 analysis, respectively. The number of tooth sites PL+ and BOP+ were treated as dichotomous data while the PD was treated as continual data. The significances of differences in PL+ and BOP+ among the groups, both at baseline and at the 180-day examinations, were assessed by means of the χ^2 analysis; when significant differences were found a further Bonferroni-corrected χ^2 analysis was performed as pairwise comparisons between the groups. Moreover, a McNemar test was used to determine the significance of differences in PL+ and BOP+ within each group over time. The significance of differences in FMPS, FMBS, PD and CAL among the experimental groups at baseline and the 180-day examinations were assessed by means of the one-way ANOVA; when significant differences were found, a Bonferroni-corrected *t*-test for independent samples was performed as pairwise comparisons between the groups. Thereafter, within the groups, the changes in FMPS, FMBS, PD and CAL over time were tested by a paired *t*-test.

Differences in the number of sites positive for each microbial species, among the groups by time point, were

tested by a χ^2 test followed by a Bonferroni-corrected χ^2 test as pairwise comparisons. Furthermore, within the experimental groups, differences in data over time were processed by a Cochran test followed by a Bonferroni-corrected McNemar test as pairwise comparisons.

A probability of $p<0.05$ was accepted for rejection of the null hypothesis.

Results

No side-effects occurred in any of the patients. Clinical outcomes over time in the different experimental groups are summarized in Table 1. At the baseline, all clinical parameters were similar among the groups, without any statistically significant differences. FMPS, FMBS and PL did not change at a statistically significant level after treatment in any of the three groups, and there were no differences among the groups at either of the clinical evaluations. BOP was significantly reduced in both the MGs and CGs after treatment, as compared to the baseline levels ($p<0.05$); in contrast, BOP did not change in the PGs throughout the study. At the 180-day examination, a statistically significant difference in BOP was recorded among the groups (MGs and CGs versus PGs, $p<0.05$). PD was significantly reduced in all three experimental groups at the end of the study as compared to the baseline levels ($p<0.01$). Although in the MGs and CGs the reduction in the mean PD throughout the study was greater than that in the PGs, the differences at the 180-day examination were not statistically significant. Finally, there was a significant reduction of CAL at the end of the study, as compared to the baseline levels, in both the MGs and CGs ($p<0.05$ and $p<0.01$, respectively), but not in the PGs. At the 180-day examination, however, a significant difference in the CAL levels was not observed among the groups.

Microbiological features carried out in this study are summarized in Table 2. At the baseline, no significant differences among the groups were detected in the frequencies of detection of each monitored bacteria. The number of significantly reduced bacterial species after treatment were 12 in the MGs, 18 in the CGs and four in the PGs; the other species remained at baseline frequencies of detection throughout the study. In the MGs and CGs, putative periodontopathic micoorganisms, such as *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia* and *V. parvula* had reduced numbers of colonies after the treatment. In the PGs, only *Eubacterium lentum*, *Fusobactreium mortiferum*, *Streptococcus mitis* and *S. sanguis* showed significant reductions in their frequencies of detection throughout the study.

Discussion

We have performed a placebo-controlled clinical trial to examine whether positive clinical effects can be obtained by means of chemical control of subgingival plaque in persistent periodontal pockets of patients affected by chronic periodontitis. The initial treatment consisted of SRP, and no open flap curettage was performed. Repeated subgingival administration of one of two different antimicrobial gels were made weekly over a 4-week period, with a PG also being administrated to the control group. In particular, the test gels contained 1% metronidazole or 1% chlorhexidine, while the PG consisted of glycerine. Overall, the frequency of detections of several bacterial species, such as *S. intermedius*, *S. mutans*, *S. salivarius* and *S. sanguis* as well as *Lactobacilli*, were lower than what would be expected. However, the SRP previously performed in each experimental site, may have contributed to these results. The results reveal that subgingival administrations of both metronidazole and chlorhexidine gels (1%) may have a role in the management of persistent pockets. However, while BOP and PD significantly decreased in the MGs and CGs after treatment, in comparison with the baseline values, PL remained at the baseline levels throughout the study in all experimental groups (Table 1). Moreover, several periodontopathogens, in the interval of monitoring, showed statistically significant lower frequencies of detection, as compared to those of the baseline (Table 2). The effects of a crevicular washout were evaluated through the PGs. In these patients, no changes in the clinical parameters were seen throughout the study, except for PD; moreover, four bacterial species significantly decreased their subgingival detection after treatment. Also of note, none of the patients underwent any side-effects during the study due to any of the periodontal treatments.

The difficulty of reaching the bottom of the pockets with the curettes can lead to the failure of SRP, resulting in persistence of periodontal pockets after treatment. In this case, a surgical approach can be indicated. It has also

Table 1. Clinical parameters at the baseline and at the 180-day examinations in the different experimental groups

	Baseline			180 days			Among groups differences	
	MG	CG	PG	MG	CG	PG	Baseline	180 days
FMPS	12.2 ± 4.4	13.5 ± 4.3	14.8 ± 4.3	13.5 ± 3.8	12.8 ± 5.2	14.2 ± 4.2	NS	NS
FMBS	7.6 ± 4.0	8.9 ± 4.6	9.3 ± 4.0	9.1 ± 3.7	7.3 ± 4.8	8.5 ± 3.5	NS	NS
PL+	12	13	17	10	11	16	NS	NS
BOP+	15	16	18	7 p,b	8 p, b	16	NS	$p<0.05$
PD	6.5 ± 0.9	6.8 ± 1.2	6.7 ± 1.4	5.0 ± 1.9b	5.1 ± 1.5 b	6.0 ± 1.9 b	NS	NS
CAL	7.1 ± 1.2	7.0 ± 1.5	6.9 ± 1.3	6.7 ± 1.3b	6.4 ± 1.6 b	6.8 ± 1.4	NS	NS

MG, 1% metronidazole gel group ($n=19$); CG, 1% chlorhexidine gel group ($n=20$); PG, placebo gel group ($n=24$); FMPS, mean values ± SDs of full-mouth plaque score; FMBS, mean values ± SDs of full-mouth bleeding score; PL+, number of experimental gingival sites positive for the presence of bacterial plaque; BOP+, number of experimental gingival sites positive for the presence of bleeding on probing; PD, mean values ± SDs of probing depth in each experimental site; CAL, mean values ± SDs of clinical attachment level in each experimental site. Results of multiple pairwise comparisons within each group between the time points: b, different from the corresponding baseline value. Results of the multiple pairwise comparisons within each time point between the groups: p, different from PG; c, different from CG. NS: no statistically significant difference.

Table 2. Number of sites positive for the presence of each bacterial species over time in the different experimental groups

	Baseline			Day 7			Day 15			Day 30			Day 90			Diff. over time		
	MG	CG	PG	MG	CG	PG	MG	CG	PG	MG	CG	PG	MG	CG	PG	MG	CG	PG
Actinobacillus actinomycetemcomitans	12	16	18	11	7	21	8 p	9 p	22	9	11	20	8 p	9 p	20	NS	$p<0.05$	NS
Diff.		NS			$p<0.01$			$p<0.01$			$p<0.05$			$p<0.01$				
Actinomyces israelii	14	17	17	13	11	19	7 p	8 p,b	21	7	8	21	7 p	8	17	$p<0.01$	$p<0.05$	NS
Diff.		NS			NS			$p<0.01$			$p<0.05$			$p<0.01$				
Actinomyces naeslundii	9	10	12	10	8	17	9	9	15	8	9	15	6	8	11	NS	NS	NS
Diff.		NS			NS			NS			NS			NS				
Actinomyces odontolyticus	10	14	12	6	5 b	14	5	3 b	11	5	9	8	5	9	9	NS	$p<0.01$	NS
Diff.		NS			NS			NS			NS			NS				
Actinomyces viscosus	5	2	6	3	0	4	4	0	4	6 c	0	5	0	0	4	NS	NS	NS
Diff.		NS			NS			NS			$p<0.05$			$p<0.05$				
Bacteroides capillosus	4	9	8	6	2	11	2	0 p,b	7	3	0 p,b	8	3	0 p,b	8	NS	$p<0.01$	NS
Diff.		NS			$p<0.05$			$p<0.05$			$p<0.05$			$p<0.05$				
Bacteroides gracilis	9	16	15	11	15	13	5 c	14	13	9	9	15	8	12	16	NS	NS	NS
Diff.		NS			NS			$p<0.05$			NS			NS				
Capnocytophaga	8	7	9	3	1	8	1 p	2	10	1	2	7	2	0	5	$p<0.01$	$p<0.01$	NS
Diff.		NS			NS			$p<0.01$			NS							
Eikenella	8	6	7	2	2	5	2	1	4	1	1	8	1	1	2	$p<0.01$	$p<0.05$	NS
Diff.		NS			NS			NS			$p<0.05$			NS				
Eubacterium lentum	6	7	8	1	1	8	1	1	7	0	1	3	0	0	4	$p<0.01$	$p<0.01$	$p<0.05$
Diff.		NS			$p<0.05$			$p<0.05$			NS			$p<0.05$				
Fusobactreium mortiferum	4	5	7	0	0	6	3	0	2	0	0	0	0	0	3	$p<0.05$	$p<0.01$	$p<0.05$
Diff.		NS			$p<0.01$			NS			–			–				
Fusobacterium nucleatum	6	7	8	2	5	6	3	9	9	4	6	9	1 p	8	10	NS	NS	NS
Diff.		NS			NS			NS			NS			$p<0.05$				
Gemella morbillorum	4	2	4	3	4	5	1	4	0	0	4	1	1	0	2	NS	NS	NS
Diff.		NS			NS			$p<0.05$			$p<0.05$			NS				
Lactobacillus acidophilus	14	16	22	8	15	17	7 p	9	20	10	13	19	9 p	13	21	NS	NS	NS
Diff.		NS			NS			$p<0.01$			NS			$p<0.05$				
Leptotrichia buccalis	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	–	–	NS
Diff.		–			NS			–			–			–				
Nocardia asteroides	0	2	1	0	0	1	0	0	2	0	1	0	0	0	1	–	NS	NS
Diff.		NS			NS			NS			NS			NS				
Peptostreptococcus micros	8	4	4	3	1	5	1	4	4	7	3	3	6	4	5	$p<0.05$	NS	NS
Diff.		NS			NS			NS			NS			NS				
Peptostreptococcus oralis	2	5	6	1	1	7	1 p	1 p	11	1 p	1 p	12	1	1	8	NS	NS	NS
Diff.		NS			$p<0.05$			$p<0.01$			$p<0.01$			$p<0.05$				
Peptostreptococcus spp	16	14	17	10	8 p	21	10c	0 p,b	13	7 c,b	0 p,b	17	9	7	15	$p<0.05$	$p<0.01$	NS
Diff.		NS			$p<0.01$			$p<0.01$			$p<0.01$			NS				
Porphyromonas asaccharolytica	0	4	6	0	3	3	1	0	3	0	0	2	0	0	0	NS	$p<0.01$	NS
Diff.		NS			NS			NS			NS			–				
Prevotella intermedia	9	15	12	10	8	11	10	9	11	12 p	7	6	11	5 b	11	NS	$p<0.05$	NS
Diff.		NS			NS			NS			$p<0.05$			NS				
Prevotella melaninogenica	13	15	19	9	7	14	8	3 p,b	16	4 p,b	5 p,b	17	7 p	9	19	$p<0.05$	$p<0.01$	NS
Diff.		NS			NS			$p<0.01$			$p<0.01$			$p<0.05$				

Table 2. (Contd.)

	Baseline			Day 7			Day 15			Day 30			Day 90			Diff. over time		
	MG	CG	PG	MG	CG	PG	MG	CG	PG	MG	CG	PG	MG	CG	PG	MG	CG	PG
Propionibacterius acnes	12	11	11	3 p	9	14	5	7	10	4	9	13	1 p,b	8	15	$p<0.01$	NS	NS
Diff.		NS			$p<0.05$			NS			NS			$p<0.01$				
Porphyromonas gingivalis	9	14	11	7	9	14	4 p	9	15	4	7	12	1 c,p	9	14	$p<0.05$	NS	NS
Diff.		NS			NS			$p<0.05$			NS			$p<0.01$				
Streptococcus costellatus	7	4	4	0	0	0	7 c	0	2	3	0	0	0	0	3	$p<0.01$	$p<0.01$	NS
Diff.		NS			–			$p<0.01$			$p<0.05$			NS				
Streptococcus intermedius	17	18	21	13	9 p,b	23	12	13	22	16	15 p	24	14 p	13 p	24	NS	$p<0.05$	NS
Diff.		NS			$p<0.01$			NS			$p<0.05$			$p<0.05$				
Streptococcus mitis	9	13	12	10 c	2 b	8	8	5	11	9	7	5	7	12	12	NS	$p<0.01$	$p<0.01$
Diff.		NS			$p<0.05$			NS			NS			NS				
Streptococcus mutans	13	16	19	12	10	20	12	6 p,b	19	11	6 p	19	14	7 p	22	NS	$p<0.01$	NS
Diff.		NS			NS			$p<0.01$			$p<0.01$			$p<0.01$				
Streptococcus salivarius	12	16	14	10	13	13	10	10	17	10	13	17	11	10	17	NS	NS	NS
Diff.		NS			NS			NS			NS			NS				
Streptococcus sanguis	14	16	20	10	6 p,b	20	10	8	18	10	4 p,b	17	9	13	13	$p<0.01$	$p<0.01$	$p<0.01$
Diff.		NS			$p<0.01$			NS			$p<0.01$			NS				
Veillonella parvula	6	11	9	2	4	5	1c	9	4	2	3	4	1	10	6	NS	$p<0.05$	NS
Diff.		NS			NS			$p<0.01$			NS			$p<0.01$				

MG, 1% metronidazole gel group ($n = 19$); CG, 1% chlorhexidine gel group ($n = 20$); PG, placebo gel group ($n = 24$). Results of multiple pairwise comparisons within each group between the time points: b, different from the corresponding baseline value. Results of the multiple pairwise comparisons within each time point between the groups: p, different from PG; c, different from CG. NS: No statistically significant difference.

been demonstrated that the experience of the operator may influence the success of such treatment in the deepest pockets (Brayer et al. 1989). Moreover, some subgingival microbiota can also be detected in soft periodontal tissues, other than subgingival plaque (Winkler et al. 1988, Sreenivasan et al. 1993), and thus persist after accurate SRP. Finally, the time spent and the numbers of sites that require instrumentation, are likely to influence the outcome (Griffiths et al. 2000). For these reasons, in the present study the same operator always carried out the treatment for a maximum of 30 min per quadrant, at 1-week intervals. Moreover, persistent pockets found in third molars and severely malpositioned teeth were also excluded (Rooney et al. 2002). The PD recorded at the baseline may also influence the clinical improvement, as previously described (Griffiths et al. 2000); hence, only persistent pockets with 5–9 mm of PD were treated by the subgingival gels. In the present study, the follow-up was up to 180 days for the clinical parameters, and up to 90 days for the microbiological evaluations. In the dental literature, as little as 6 weeks has been used (Noyan et al. 1997), but more commonly studies are of 6 months (Pedrazzoli et al. 1992) or 9 months (Steltzel & Flores-de-Jacoby 2000). Indeed, the period needs to be sufficiently long to allow clinically evident improvements to occurr, with the necessity of it being related to the practical timing of re-examination and re-treatment (Griffiths et al. 2000). Furthermore, considering that the ideal time of recall in supportive periodontal treatment is 90 days, in the present study microbial change parameters were monitored at a similar time interval. Therefore, if beneficial microbial changes occur as a consequence of subgingival gel administration up to a 90-day follow-up, this could indicate that repeating this therapy in coincidence with each recall visit in clinical practice could be carried out. On the contrary, other studies examining adjunctive chemotherapeutic treatment to SRP have used microbiological evaluations and reported that the maximum benefits are seen within the first 90 days post-treatment (Rooney et al. 2002). Hence, a follow-up after 180 days would be probably irrelevant to the adjunctive action, and any changes would be independent of the antimicrobial action (Rooney et al. 2002).

In the present study both the FMPS, FMBS were always less than 20% and similar among the three groups at each clinical examination with no significant changes over time in each group (Table 1). This was probably due to the repeated OHI given to all participants. Similarly, the site-based clinical examinations show that PL scores did not change over time for any of the experimental groups, and that no significant differences were detected among them either at the baseline or at the 180-day examination (Table 1). These results are supported by the fact that no mechanical actions are provided by the subgingival gels on the supragingival plaque. Therefore, it appears that the subgingival administration of gels does not influence the supragingival plaque accumulation. It has been reported that recording the BOP as dichotomous data may not be properly accurate (Griffiths et al. 2000). Indeed, a site which had profuse

bleeding at the baseline and which, following the treatment, is reduced to a single point of bleeding has shown considerable improvement; however, in the dichotomous system this would represent no change as the site bled at both examinations (Griffiths et al. 2000). On the other hand, the advantage offered by this system is to allow comparisons with other studies. In spite of these considerations, our results obtained for the BOP show that while the MGs and CGs underwent significant reductions of the bleeding sites throughout the study, the same was not observed for the PGs. This may be related to the antimicrobial actions of the MGs and CGs that may have reduced the pathogenicity of the subgingival plaque. Indeed, several studies have demonstrated that both metronidazole and chlorhexidine administrated subgingivally, as an alternative to SRP, can lead to a significant improvement in the clinical parameters in patients affected by chronic periodontitis (Pedrazzoli et al.1992, Unsal et al. 1995). However, our clinical findings cannot be compared with any previous ones; indeed, although Salvi et al. (2002) evaluated the clinical healing obtained after chemotherapeutic treatments of persistent periodontal pockets in patients affected by chronic periodontitis, they did not report any indications concerning the PL or BOP scores following the treatment.

In the present study, PD was significantly reduced following the treatments in each experimental group (Table 1). However, while the mean gain for the MGs and CGs was higher than 1 mm, in the PGs it was lower than 1 mm (Table 1). It can be proposed that although a statistically significant improvement was recorded for each group, it was also clinically relevant for the MGs and CGs only. Indeed, routine oral hygiene has been described as reducing PD by about 0.2 mm by itself (Cutler et al. 2000); therefore, the data regarding the PD in the PGs should be interpreted with caution considering that each patient received accurate OHI at the beginning of the study. Moreover, with chronic periodontitis it has been reported that clinical improvement after treatment may depend upon the severity of the disease, with the lower level of disease leading to less margin for differences to be detected (Palmer et al. 1998, Salvi et al. 2002). The gain of the PD obtained in the present study in the CGs and MGs is greater than that obtained by Salvi et al. (2002). In particular, they found that gains of the PD were always less then 1 mm for all the tested groups. These discrepancies may be explained by the differences in the protocols of subgingival administration used; indeed, in the present study, the treatment was repeated four times at 1-week intervals, while Salvi et al. (2002) used one and two administrations of PerioChip® and Elyzol® Dental Gel, respectively. The different formulations of the chemotherapeutic agents should also be considered. Finally, in the present study, neither current nor former smokers were included, as opposed to the clinical trial performed by Salvi et al. (2002).

The CAL levels were significantly reduced in the MGs and CGs only (Table 1). The considerations listed above for the PD can also explain these results. Interestingly, the lack of significant improvement in terms of CAL in the PGs could indicate that the gain in the PD observed in these patients is probably due to the reduction in a gingival enlargement as a consequence of oral hygiene practices, rather than beneficial effects provided by the administration of the placebo.

To date, there are a number of studies that have evaluated the benefits obtained by adjunctive chemical treatment to non-surgical therapy in the management of periodontal diseases through microbiological analyses (Lie et al. 1998, Griffiths et al. 2000, Steltzel & Flores-de-Jacoby 2000). However, controversial results have been seen in terms of microbiological findings; this may be due to the different methods for plaque sampling or culturing (Rooney et al. 2002). Indeed, culture methods can lead to underestimations of numbers of some bacteria, while molecular methods reveal higher counts (Rooney et al. 2002). The number of the patients included in a study can also influence the microbiological observations (Rooney et al. 2002).

Riep et al. (1999) have reported that a combination of SRP plus 25% metronidazole gel administered subgingivally has comparable effects on the subgingival microflora as compared to SRP alone. Rudhart et al. (1998) have observed that the subgingival administration of 25% metronidazole gel alone can lead to the same microbiological outcome as compared to SRP. Other studies have reported a more favourable effect when 25% metronidazole gel is combined with SRP, as compared to SRP alone. The results obtained in the present study are supported also by a previous report by Piccolomini et al. (1999a); first, they evaluated the effects of subgingival administration of 1%, rather than 25%, metronidazole gel as an alternative to SRP, reporting a significant reduction in several subgingival periodontopathogens. In the present study, of the 31 bacterial species cultured from the subgingival plaque samples, up to 12 underwent a significant reduction in the MGs after treatment (Table 2). In particular, several periodontopathogens, including *Actinomyces israelii*, *Capnocytophaga*, *Eikenella*, *P. gingivalis*, *P. melaninogenica* and *P. micros*, reduced their counts in this group. These results may disagree with those of Salvi et al. (2002), who reported unsatisfactory results in terms of culture counts after treatment with Elyzol® Dental Gel in persistent periodontal pockets. This difference may be explained as detailed above for PD. The major advantages of this kind of treatment are the lack of undesirable effects, such as dental pigmentation produced by chlorhexidine, and its very low price as compared to the 25% concentration formulations.

Previous studies have tested the efficacy of chlorhexidine at a 1% concentration in the treatment of chronic periodontitis. Unsal et al. (1995) have reported that clinical and microbiological benefits can be achieved when subgingival administration of CG is an adjunct to SRP, as compared to SRP alone. Similar results were obtained by Fine et al. (1994) regarding subgingival irrigation of chlorhexidine. Piccolomini et al. (1999b) have reported clinical and microbiological benefits after subgingival administration of CG as an alternative to SRP. Finally, Salvi et al. (2002) have reported that the use of PerioChip® may be useful in reducing the levels of some periodontopathogens in residual pockets, and that such treatment does not eliminate the need for a surgical approach. In the present study, 18 bacterial species from the subgingival plaque of the CGs underwent a remarkable reduction in their frequency of subgingival detection. In particular, several periodontopathogens reduced in the CGs were the same as those in the MGs. Through this evidence, and as for the 1% metronidazole gel, the 1% chlorhexidine gel

administered subgingivally can be considered useful in the management of residual periodontal pockets after SRP during chronic periodontitis. These pharmacological treatments may also be useful for those patients who have contra-indications for surgical treatment. Finally, in the present study a complete eradication of several of the monitored bacteria in the MG and CG groups was not observed, although significantly better clinical findings were seen. This result is in accordance with those reported by Salvi et al. (2002).

In the PGs, *E. lentum*, *F. mortiferum*, *S. mitis* and *S. sanguis* underwent significant decreases in their relative counts after treatment, as compared to the baseline values. These results may be supported by the washout exercised by the PG within the periodontal pockets. In this regard, Cutler et al. (2000) have reported that subgingival water irrigation plus OHI given to the patients have a more favourable clinical benefit as compared to OHI only. It may also be proposed that some of the microbiological features observed in the MGs and CGs could have been in part due to the washout, as this result from the PGs indicates. From these considerations, the clinical relevance of this PG seems not to be sound.

Briefly, the present study has initially demonstrated the effectiveness of repeated subgingival administrations of 1% metronidazole and 1% chlorhexidine gels in producing clinically and microbiologically relevant benefits in persistent periodontal pockets of patients affected by mild-to-severe chronic periodontitis. However, no complete eradication of periodontopathogens was obtained in either the CGs or the MGs. Further studies are needed to clarify this aspect.

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