Efficacy of Chlorhexidine, Metronidazole and Combination Gel in the Treatment of Gingivitis - A Randomized Clinical Trial

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Abstract

Objective: Effective plaque control is essential for prevention of gingivitis and periodontitis. The aim of this 24-week follow-up parallel study was to evaluate the efficacy of three topical gels in the treatment of gingivitis as compared to placebo gel. **Methods**: One hundred twenty subjects diagnosed with chronic generalized gingivitis were selected and randomly divided into four groups: Group 1 – placebo gel, Group 2 – chlorhexidine (CHX) gel, Group 3 – metronidazole (MTZ) gel and Group 4 – chlorhexidine-metronidazole (CHX-MTZ) gel. Clinical evaluation was undertaken using the gingival index (GI) of Löe and Silness and the plaque index (PI) at baseline, 6 weeks, 12 weeks and 24 weeks. Microbiological analysis was also done at the same time intervals. A subjective evaluation was also undertaken by questionnaire. **Results**: Groups treated with all three gels (CHX, MTZ and CHX-MTZ) showed significant clinical and microbiological improvement as compared to the group treated with a placebo gel. The reduction in PI, GI and microbiological count in the group treated with the CHX-MTZ combination gel was significant when compared to those treated with CHX and MTZ gels. **Conclusion**: Topical application of CHX or MTZ alone or in combination may have a role in the management of gingivitis.

Key words: chlorhexidine, clinical trial, gingivitis, metronidazole

Introduction

Periodontal diseases are infections initiated by bacterial biofilms that form on the surfaces of teeth in close proximity to the supporting tissues. The susceptibility to periodontitis is influenced by many factors such as smoking, diabetes and genetics, and prevention of gingival inflammation prevents periodontitis (Kinane and Attstrom, 2005). Periodontal disease can be prevented by maintaining effective plaque control at home (Sheiham, 1997). The rationale for the use of antiplaque agents as adjuncts to mechanical cleaning methods is based on two factors. First, plaque is the major etiological factor in gingivitis (Syed and Loesche, 1978). Second, the prevalence of gingivitis and evidence from studies suggest that mechanical cleaning methods are inadequate (De La Rosa *et al.*, 1979; Macgregor and Rugg-Gunn, 1979; Addy *et al.*, 1987).

Chlorhexidine (CHX) remains the gold standard of chemical antiplaque agents and remains one of the most effective topical antiseptics reported to date that has been successfully used for treating plaque-related gingivitis (Schiott *et al.*, 1970; Löe and Schiott, 1970; Quirynen, 2001). Chlorhexidine has been reported to have some reversible local side effects, such as staining of the teeth and tongue and desquamation of the oral mucosa. Staining is largely dose-dependent, whereas desquamation of the oral mucosa and perturbation of taste is largely concentration-dependent (Brecx *et al.*, 1993).

Metronidazole (MTZ) has antibacterial effects primarily exerted on Gram-positive and Gram-negative obligate anaerobes (Goodson, 1994). Some studies have tested the efficacy of systemic MTZ (Palmer *et al.*, 1998; Noyan *et al.*, 1997) during periodontal disease while others have tested the topical application of MTZ directly into the infected pocket either alone (Pedrazzoli *et al.*, 1992; Stelzel, 1997) or as an adjunct to mechanical debridement (Awartani *et al.*, 1998; Riep *et al.*, 1999).

To date, there is no study comparing the topical effects of CHX gel, MTZ gel and the combination of CHX and MTZ gel in gingivitis subjects. This study is

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designed to compare the efficacy of topically applied CHX, MTZ and the combination of these two gels over a period of 24 weeks in subjects with gingivitis.

Materials and methods

After ethical approval was granted by the Institutional Ethical Committee and Review Board of the Government Dental College and Research Institute, Bangalore, 132 dentate subjects (67 males and 65 females who reported to the Department of Periodontics, Government Dental College and Research Institute, Bangalore) were recruited for this double-blinded, parallel, randomized, controlled clinical trial conducted from June to December 2011. All randomly screened participants were informed about the nature of the study and signed an informed consent form. Group sample sizes were decided by power analysis with 95% power and a significance level of 0.05.

Subjects diagnosed with chronic generalized gingivitis, aged 25–40 years and having at least 20 natural teeth, with no history of periodontal therapy or previous use of antibiotics or anti-inflammatory medication within the preceding six months, were included in the study. All patients fulfilled the clinical criteria of a gingival index (GI; Löe and Silness, 1963) > 1, probing depth ⩽ 3 mm and clinical attachment loss = 0, with no evidence of radiographic bone loss. Subjects with known allergies to the constituents of the formulation, haematological disorders or other systemic illnesses, alcoholics, immunocompromised subjects, pregnant or lactating females, subjects undergoing orthodontic treatment and subjects who used tobacco in any form were excluded.

Each subject was randomly assigned by a computer-generated numbering sequence to one of the four groups (33 subjects in each group). Placebo gel (Charak Pharmaceuticals, Bangalore, India); CHX gel (1% w/w; ICPA, Mumbai, India); MTZ gel (10 mg; Lekar Pharmaceuticals, Mumbai, India) and CHX-MTZ gel (CHX [0.25% w/w] and MTZ [10 mg]; Indoco (Warren), Mumbai, India).

Patients accepted to participate in the study returned for a baseline examination. Patients were told not to perform any oral hygiene (including chewing gum) for eight hours prior to the baseline and follow-up examinations. Patients were assessed for plaque using the plaque index (PI) using the Tureskey modification of the Quigley Hein Index (Quigley and Hein, 1962; Tureskey *et al.*, 1970) and gingival inflammation using the GI (Löe and Silness, 1963). Following the assessments, all subjects received scaling and prophylaxis to remove plaque, calculus and extrinsic stain.

The gels were dispensed to subjects by a dental assistant not involved in the study. All tubes had a plain white covering labeled only with lot numbers to ensure proper blinding of the product from the patients and the examiner. Subjects were instructed to apply a pea-sized amount of gel gently with the index finger to the gums an hour after regular brushing and to leave it for five minutes before rinsing. Subjects were also asked to refrain from all other unassigned forms of oral hygiene aids, including dental floss, chewing gum or oral rinses during the study. No oral hygiene instructions such as brushing and flossing were given to the patients to exclude the influence of improved oral hygiene practices on the results.

The clinician, who was blinded to the gels assigned to the subjects, conducted all the examinations and scorings. Subjects were assessed for GI and PI in the same dental unit under identical conditions at baseline, 6 weeks, 12 weeks and 24 weeks. Intra-examiner calibration was performed on 20 patients before the study and the intra-examiner agreement was 95.2% (κ = 0.905).

Apart from the clinical evaluation, a subjective evaluation was also undertaken at each visit using a questionnaire relating to the taste and flavour of the gels or any adverse effect experienced after use. To check for compliance, the participants were asked to return their assigned gel tubes so that the investigator could verify the amount of gel that was used.

At the baseline and at each visit, a dental plaque sample was collected from each subject. Each volunteer was asked to gargle with saline to remove any food debris. The plaque was collected from the buccal groove of the lower first molar tooth using a sterile paper point such that the standardized length of the paper point (colored area) touched the tooth for 5 seconds. This specimen was immersed in 1 ml of phosphate buffered saline (PBS). These plaque specimens were vortexed for 10 sec and immediately subcultures were performed on Mitis Salivarius (MS) agar for streptococcus species and gelatin-metronidazole-cadmium medium (GMC) for *Actinomyces* species.

Colonies of *Streptococcus sanguis*, *S. mitis*, *S. intermedius, S. oralis*, *Actinomyces viscosus* and *A. naeslundii* were identified based on colony morphology. Colonies with similar morphology were counted using a colony counter: the numbers were recorded and the total microbial count was taken into account.

Statistical analysis

Analysis of data was carried out using SPSS 10.5 (SPSS version 10.5, SPSS, Chicago, IL). The values of different parameters collected are expressed as mean ± standard deviation (SD). Normality of continuous data was tested using the Kolmogorov Smirnov test. Mean changes from baseline to 6 weeks, 12 weeks and 24 weeks were also calculated. Comparisons among the four treatment groups and within each treatment group were performed using one-way ANOVA.

Table 1. Plaque index scores, gingival index scores and microbiological counts for all groups at different follow-up visits

Parameter	Group	Baseline	6 wks	12 wks	24 wks
PI	Placebo	4.31 ± 0.45	4.19 ± 0.45	4.03 ± 0.47	4.06 ± 0.46
	CHX	4.52 ± 0.44	3.54 ± 0.35	2.96 ± 0.26	2.48 ± 0.42
	MTZ	4.48 ± 0.44	3.94 ± 0.39	3.03 ± 0.34	2.71 ± 0.32
	CHX-MTZ	4.54 ± 0.44	3.25 ± 0.41	2.45 ± 0.43	2.13 ± 0.38
GI	Placebo	1.84 ± 0.30	1.79 ± 0.32	1.68 ± 0.31	1.65 ± 0.29
	CHX	1.89 ± 0.32	1.24 ± 0.33	1.12 ± 0.32	0.86 ± 0.33
	MTZ	1.86 ± 0.31	1.43 ± 0.27	1.22 ± 0.33	0.99 ± 0.30
	CHX-MTZ	1.87 ± 0.33	1.01 ± 0.38	1.06 ± 0.36	0.51 ± 0.29
Microbial	Placebo	33.36 ± 1.59	33.00 ± 1.26	32.50 ± 1.01	32.20 ± 1.32
counts	MTZ	33.13 ± 0.94	23.33 ± 2.14	19.53 ± 1.20	14.37 ± 1.50
(x 10^4)	CHX	33.43 ± 1.19	21.37 ± 1.69	13.33 ± 2.07	9.37 ± 1.50
	CHX-MTZ	33.23 ± 1.52	17.27 ± 1.74	10.33 ± 1.37	6.63 ± 1.50

CHX, chlorhexidine; CHX-MTZ, chlorhexidine-metronidazole combination; GI, gingival index; MTZ, metronidazole; PI, plaque index

Results

Twelve subjects did not complete the study and were excluded from the analysis. There were no significant differences among the groups with respect to any parameter at baseline. There was a gradual decrease in the PI and GI scores in all the groups over a period of 24 weeks (*Table 1*).

A significant reduction in mean PI was observed in all groups at all time intervals except in the placebo group between 12 and 24 weeks. A significant reduction was also observed in GI for all groups between the 6-week and 12-week time intervals and between the 12-week and 24-week time intervals except in the placebo group. There was no significant reduction in GI between any time intervals in the placebo group.

Microbial counts also showed significant reduction in all groups at all time intervals except in the placebo group (*Tables 2 and 3*).

On subjective evaluation, about 21% of subjects reported an unpleasant taste and discolouration of teeth following the use of CHX gel and 3% with CHX-MTZ gel.

Discussion

The purpose of this study was to assess and compare the clinical and microbiological effects of CHX, MTZ and a combination of these two gels applied over a period of 24 weeks in subjects with gingivitis. All three treatment gel groups (CHX, MTZ and CHX–MTZ) showed significant improvement in clinical and microbiological parameters compared to the placebo gel group.

The predominant Gram-positive species associated with gingivitis include *S. sanguis*, *S. mitis, S. intermedius*, *S. oralis*, *A. viscosus* and *A. naeslundii* (Moore and Moore, 1994). Therefore, these organisms were specifically cultured to assess the microbiology.

CHX is considered as the gold standard (Schiott, 1970) antiplaque agent because of its substantivity and is used as a positive control in the present study. CHX has a wide spectrum of activity encompassing Gram-positive and Gram-negative bacteria. In the current study, a significant reduction in PI and GI scores at all time intervals was noticed with the use of both CHX and CHX-MTZ gels, and a significant reduction in microbial counts also was found. CHX acts on the cell walls of the microorganisms by changing their surface structures (Brecx and Theilade, 1984).

In the current study there was significant reduction in PI, GI and microbial counts in the MTZ group as compared to the placebo group. It is known that cytotoxic metabolites of MTZ directly interact with bacterial DNA, and possibly other macromolecules, resulting in cell death (Goodson, 1994). Studies have shown that CHX gel is significantly more active than placebo, or a control substance, in controlling plaque in different patient groups (Löe and Schiott, 1970; Quirynen *et al.*, 2001)

Lander *et al.* (1986) investigated the impact of an

Table 2. Mean change from baseline in plaque index and gingival index scores at different follow-up visits

Parameter	Group	Baseline - 6 wks	Baseline - 12 wks	Baseline - 24 wks
PI	Placebo	0.12 ± 0.20	0.28 ± 0.22	0.25 ± 0.19
	CHX	0.98 ± 0.54	1.56 ± 0.47	2.04 ± 0.66
	MTZ	0.54 ± 0.27	1.45 ± 0.50	1.77 ± 0.48
	CHX-MTZ	1.29 ± 0.60	2.09 ± 0.49	2.41 ± 0.59
GI	Placebo	0.05 ± 0.55	0.16 ± 0.48	0.19 ± 0.45
	CHX	0.65 ± 0.36	0.77 ± 0.33	1.03 ± 0.46
	MTZ	0.43 ± 0.35	0.64 ± 0.47	0.87 ± 0.38
	CHX-MTZ	0.86 ± 0.40	0.81 ± 0.39	1.36 ± 0.46
Microbial counts (x 10^4)	Placebo	0.36 ± 2.09	0.53 ± 1.61	0.73 ± 1.55
	CHX	12.06 ± 1.74	20.10 ± 1.99	24.07 ± 1.53
	MTZ	9.8 ± 2.61	13.6 ± 1.59	18.77 ± 1.74
	CHX-MTZ	15.96 ± 2.30	22.90 ± 1.86	26.60 ± 2.44

CHX, chlorhexidine; CHX-MTZ, chlorhexidine-metronidazole combination; GI, gingival index; MTZ, metronidazole; PI, plaque index

Table 3: Intra-group comparison of change from baseline at various follow-up visits

Parameter	Group	6 wks vs 12 wks (*p*-value)	6 wks vs 24 wks (*p*-value)	12 wks vs 24 wks (*p*-value)
PI	Placebo	0.004*	0.011*	0.568
	CHX	< 0.001*	< 0.001*	0.002*
	MTZ	< 0.001*	< 0.001*	0.014*
	CHX-MTZ	< 0.001*	< 0.001*	0.027*
GI	Placebo	0.690	0.549	0.973
	CHX	0.184	< 0.001*	0.014*
	MTZ	0.056	< 0.001*	0.043*
	CHX-MTZ	0.600	< 0.001*	< 0.001*
Microbial counts (x 10^4)	Placebo	0.725	0.725	0.725
	CHX	< 0.001*	< 0.001*	< 0.001*
	MTZ	< 0.001*	< 0.001*	< 0.001*
	CHX-MTZ	< 0.001*	< 0.001*	< 0.001*

*Statistically significant difference. CHX, chlorhexidine; CHX-MTZ, chlorhexidine-metronidazole combination; GI, gingival index; MTZ, metronidazole; PI, plaque index

irrigant on clinical and microbiological parameters by randomly irrigating non-debrided pockets with a single dose of 0.2% CHX gel, 0.2% CHX solution or physiological saline. There were no differences between sites treated with the gel or the solution at any time.

Recently, a study has shown that 0.2 % CHX gel therapy may be an option to treat and prevent gingivitis and reduce yeast counts in children infected with HIV (Machado *et al.*, 2011). Pannuti *et al.* (2003) indicated that the use of a 0.5% CHX gel was effective in reducing interdental gingival bleeding in special patients as compared to a placebo gel. The improvement seen in the current study in the CHX gel group was in accordance with the previous studies.

The reduction in plaque and gingivitis scores in the placebo group can be attributed to the Hawthorne effect (Jeffcoat, 1992).

Several studies have tested the topical application

of MTZ directly into the infected pocket either alone (Pedrazzoli *et al.*, 1992; Stelzel *et al.*, 1997) or as an adjunct to mechanical debridement (Awartani and Zulqarnain, 1998; Riep *et al.*, 1999).

In one study MTZ administered via the systemic route during a 28-day period was found to effectively decrease plaque and gingivitis development in dogs (Heijl and Lindhe, 1979). Riep *et al.* (1999) demonstrated that there were no significant differences between treatment with MTZ dental gel plus scaling and root planing (SRP) and SRP alone.

In a study that compared a simplified oral hygiene regime (SRP and Bass brushing) with this same regime plus 0.02% CHX, 0.05% MTZ and inactive control solutions delivered supra-gingivally by a pulsating water jet irrigator, significant improvements were seen in all parameters for all the groups. CHX was found to be better in reducing PI at all times except at day 84. Although the differences were statistically highly significant, clinically the differences between groups were relatively small, except for a CHX effect on PI (Aziz-Gandour *et al.*, 1986).

Recently, a study comparing the clinical and microbiological effects of 1% CHX gel, 1% MTZ gel, and placebo gel in persistent pockets concluded that probing depth was significantly reduced by the same amount in all groups, although mean pocket reductions were greater in the CHX and MTZ group in comparison to the placebo (Perinetti *et al.*, 2004).

In accordance with the previous study, the current study also showed no significant differences in PI and GI between the CHX and MTZ gel groups at the 12-week and 24-week time intervals, indicating that the MTZ gel is equally as efficacious as the CHX gel. However, the combination gel (CHX-MTZ) group showed a significant reduction in PI, GI and microbial counts when compared to the CHX and MTZ gel groups, indicating that the combination gel demonstrates an additive effect of both components.

In the current study 21% of subjects reported an unpleasant taste and discolouration of teeth following the use of CHX gel. Three percent of those using the combination gel reported the same thing. The tooth staining is thought to be the result of a local precipitation reaction between tooth-bound CHX and chromogens found within foodstuffs and beverages (Brecx *et al.*, 1993).

Conclusion

The current study has shown that topical CHX gel, MTZ gel and combination gel (CHX-MTZ) can reduce PI, GI and microbiological counts over a period of 24 weeks. Moreover, the combination gel has been shown to be more efficacious than the CHX gel, which is the gold standard of antiplaque agents. However, topical gel application cannot be a substitute for mechanical plaque control. Further long-term studies are required to see whether the use of a topical gel will eventually result in a clinically relevant reduction in chronic periodontitis.

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