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Local Delivery of Metronidazole and Chlorhexidine as Toothpaste in Treatment of Adult Periodontitis

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Abstract: The present study was done to assess the effect of topically-applied toothpaste containing metronidazole (MTZ) and chlorhexidine (CHX) alone or in combination as an adjunctive non-surgical therapy in patients with Adult Periodontitis (AP). Eighty adult patients (50 females and 30 males, mean age of 35 years) with chronic moderate to advanced periodontitis participated in this trial. Following baseline clinical examination, including assessments of Plaque Index (PI), PPD, Clinical Attachment Level (CAL), Gingival Index (GI) and Bleeding on Probing (BOP), careful oral hygiene was instructed. The patients were randomly assigned to one of four treatment groups; placebo (toothpaste without antibacterial agents), MTZ 1%, CHX 0.2% and combined toothpaste of MTZ 1% and CHX 0.2%. Scaling and Root Planning (SRP) was done in two sessions one week before investigation. Clinical re-examination was performed at the end of week 2-4 after application of toothpaste. At the baseline the Mean±SD of PI for placebo (control treatment) and combined treatment group were 1.62 (0.83) and 1.62 (0.6) and after 4 weeks of treatment were 1.42 (0.7) and 0.85 (0.52), respectively ($p<0.001$). The improvement in CAL of drug receiving subjects was greater than that of placebo ($p = 0.002$). Combined treatment group showed more improvement in PI, GI and BOP in compared with other groups. According to the results, for improving the parameters, combination of MTZ 1% and CHX 0.2% in toothpaste composition could be more effective than each of them alone.

Key words: Toothpaste, metronidazole, chlorhexidine, adult chronic periodontitis, combination therapy

INTRODUCTION

It is commonly accepted that periodontitis is an infectious disease that occurs in susceptible individuals. Various forms of periodontal disease, e.g., aggressive periodontitis, chronic periodontitis, necrotizing periodontitis, are associated with certain groups of bacteria housed in subgingivally located biofilms (Socransky and Haffajee, 1994). Application of antibiotics into the pockets, provides an effective concentration of the active agent in the involved site with the low risk of systemic administration side effects (Henke *et al*., 2001; Quirynen *et al*., 2000; Stanford, 2001). Chlorhexidine gluconate is a product which can be used in a cross-linked, natural jelly vehicle or other polymeric base in the periodontal pocket. After the initial peakof chlorhexidine (CHX) in crevicular fluid (Soskolne *et al*., 1998), there is a 9 day minimum inhibitory concentration for more than 99% of periodontal pocket (Cetin *et al*., 2004).

Clinical efficacy of CHX has been proved unequivocally in multi-centre phase 3 trials in Europe (Soskolne *et al*., 1997) and the United States (Jeffcoat *et al*., 1998; Norkiewicz *et al*., 2001). It is suggested that adjunctive, controlled release CHX, may reduce periodontal surgery with less additional cost (Henke *et al*., 2001). Among the other locally administered adjunctive antimicrobials for AP one of the most effective agent metronidazole (MTZ) (Bonito *et al*., 2005; Ehmke *et al*., 2005).

A 12 month study on previously treated chronic periodontitis, suggested that the MTZ has positive effects on the clinical parameters and microbiological composition of subgingival plaque on new and recurrent pockets (Zee *et al*., 2006). Systemic administration of combined MTZ with amoxicillin showed a significant antimicrobial effect in patients with AP compared to receiving Scaling-Root Planning (SRP) alone (Lopez *et al*., 2006). Local use of the antimicrobials in toothpaste not

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only can be comfortable in application for patients, but also can decrease risk of side effects following systemic administration. Because, there is no other study to determine the potential value of CHX and MTZ toothpastes in treatment of Iranian peridontal patients, the aim of this trial was to evaluate the efficacy of CHX and MTZ toothpastes alone or in combination as an adjunctive therapy, immediately following the initial treatment phase (SRP) in patients with moderate to advanced chronic periodontitis.

MATERIALS AND METHODS

This study was a one month randomized, double-blinded comparative controlled-clinical trial on four groups. Before starting the investigation Scaling and Root Planing (SRP) was done for all subjects in two sessions and in a one week interval. The trial was done in the Periodontology and Pharmacology departments at Babol Medical University (Iran), 2006-2007.

Study design: Eighty adult patients (50 females and 30 males, average age of 35 years) with moderate to severe chronic periodontitis were randomly selected from the pool of patients of the Department. Inclusion criteria were: signed consent form after verbal communication and a minimum of 10 natural uncrowned teeth. All patients had to present at least 8 periodontally involved sites with minimum one Probing Pocket Depth (PPD) of > = 5 mm and Bleeding on Probing (BoP). All patients with aggressive periodontitis, history of systemic antimicrobial therapy within 2 months prior to therapy, allergy to chlorhexidine (CHX) and metronidazole (MTZ), chronic diseases such as diabetes, smoking; history of periodontal surgery, scaling and rootplanning during 3 months prior to the baseline were excluded from the study.

Toothpastes preparation: A standard toothpaste formulation as base of the preparations (Pooneh, Tehran, Iran) was used in this investigation. Toothpaste containing 0.2% CHX (chlorhexidine hydrochloride, Daru Pakhsh, Tehran, Iran) and 1% MTZ (metronidazole, Daru Pakhsh, Tehran, Iran) were prepared with the base. Four series of toothpaste were made. These included: toothpastes without drug as placebo, toothpaste containing 0.2% CHX, toothpaste containing 1% MTZ and toothpastes of CHX and MTZ as a combined preparation. All preparations were made in the lab and were separately encoded and packaged in special boxes provided at the Pharmacology Department.

Treatments: This study was designed with minor modification based on the previous investigation on CHX efficacy (Soskolne *et al*., 1997). A supragingival ultrasonic scaling and a prophylaxis were performed for all teeth before starting the investigation and all target sites were root planed under local anaesthesia for a maximum of 5 min for each tooth. One week before starting the investigation all subjects underwent scaling and root planning procedure in two sessions. Clinical examinations were Plaque Index (PI), PPD, clinical attachment level (Cal), Gingival Index (GI) and BoP. Careful oral hygiene instruction was given. The patients were then randomly assigned to one of the four treatment groups; placebo (toothpaste without antibacterial agents), MTZ 1%, CHX 0.2% and combined toothpaste of MTZ 1% and CHX 0.2%. The patients were recommended to apply the toothpaste with bass brushing method three times daily after meal. Clinical re-examination was performed at the end of week 2-4 after application of toothpaste. Through out the study all the measurements and instrumentations were performed by one person.

Clinical measurements: The following clinical measurements were recorded for the target sites (teeth No. 3, 9, 12, 19, 25, 28).

Plaque examination: Plaque burden was recorded using the Silness and Löe Plaque Index (PI) (Silness, 1964).

Probing Pocket Depth (PPD): Probing depths were measured using a manual University of North Carolina periodontal probe. Each pocket was probed 2Å to provide replicate measurements. A third measurement was made if there was a >0.5 mm difference between the first 2 recordings. The median of the 2 (or 3) recordings was used in the data analysis.

Bleeding on Probing (BOP): Bleeding from each site was assessed 25 sec after probing pocket depth using the BoP Index of Muhlemann (1977).

Clinical Attachment Level (CAL): Measurements were recorded from the cementoenamel junction (CEJ). Again, a first pass of all sites were performed and then a second reading obtained (double pass technique). The median of the 2 scores was recorded. If these varied by >0.5 mm a third reading was taken and the median of the 3 scores used for the analysis (Namgung and Yang, 1994).

Data and statistical analysis: Data were analyzed on an intention-to-treat basis with the subject as the unit of statistical analysis. The protocol-defined primary outcome

variable was the reduction of PI from baseline. Changes of PPD,BOP and CALin each site were investigated at baseline 1, 2 and 4 weeks after therapy. Overall mean of the differences from baseline were calculated for each group. Summary of statistics were determined and 4 sample ANOVA post-hoc tukey tests were undertaken to identify statistically significant differences between the treatments. Repeated Measure test was used for inter-groups data at different time points. For the distribution-free scores at the 1 month visit, nonparametric Kruskall-Wallis H-test was applied to compare data across the treatments. differences with $p<0.05$ was considered significant.

RESULTS

All of the 80 (50 females and 30 males, mean age of 35 years) recruited subjects completed the study. The ratio of male/female was 0.6. During the trial nobody reported any oral symptoms. Baseline data of placebo and drug treatment groups analyzed on a subject-wise basis which has been shown in Table 1. These data confirm that PI, GI, BOP, PPD and CAL measurements in drug and placebo groups were very close to each other at the base line.

Plaque Index (PI): No significant difference were observed at base line (Table 2). All treatment groups showed significant differences between $Pi_{baseline}$ and $PI_{week\ 4}$ ($p<0.05$). Inter-group comparative analysis showed a significant decrease between placebo and CHX alone at week 4 ($p<0.001$), placebo and MTZ+CHX at week 3 ($p = 0.013$) and week 4 ($p = 0.016$) but the effect of MTZ toothpaste compared to placebo didn't show significant difference during the study ($p = 0.228$) (Table 3).

Gingival Index (GI): GI evaluation between all groups showed significant differences at the end of week 4 in compared baseline. MTZ improved the GI at week 3 ($p = 0.014$) and week 4 ($p = 0.045$) more than placebo. The combination of MTZ and CHX had shown significant differences compared to placebo, as well ($p_{week\ 3} <0.001$, $p_{week\ 4} <0.0001$, Table 4).

Bleeding on Probing (BoP): All evaluated sites showed bleeding on probing at baseline (Table 5). At 2, 3 and 4-in re-examination intervals the drug groups demonstrated statistically significant lower bleeding score compared to placebo group (CHX at week 4 ($p<0.05$), MTZ at week 3 ($p = 0.006$) and week 4 ($p<0.0001$) and combination of MTZ + CHX at week 2 ($p = 0.013$), week 2 ($p<0.001$) and week 4 9 ($p<0.0001$).

Probing Pocket Depth (PPD): Baseline Mean±SD of PPD in MTZ, CHX and MTZ+CHX treatment sites were 2.82±0.83, 2.88±0.82 and 2.96±0.75 mm, respectively. At the end of week 4 mean PPD, in MTZ+CHX group showed

Table 1: Mean±SD of baseline data for placebo and drug treatment groups analyzed on a subject-wise basis

Groups	PI	BoP	GI	PPD	CAL
Placebo (control, n = 20)	1.62±0.83	1.76±0.59	1.87±0.52	2.84±0.83	2.64±0.78
Metronidazole 1% (n = 20)	1.53±0.54	1.49±0.64	1.74±0.56	2.82±0.83	2.66±0.82
Chlorhexidine 0.2% (n = 20)	1.70±0.58	1.68±0.67	1.82±0.55	2.88±0.82	2.70±0.75
Metronidazole+chlorhexidine (n = 20)	1.62±0.60	1.47±0.68	1.75±0.58	2.96±0.75	2.96±0.88

Table 2: Mean±SD of plaque index at baseline and changes at 2, 3 and 4 weeks compared to baseline

	PI*				
Groups	PI 0	PI 2	PI 3	PI 4	p-value **
Placebo (control, n = 20)	1.62±0.83	1.40±0.17	1.41±0.74	1.42±0.70	0.0080
Metronidazole 1% (n = 20)	1.53±0.54	1.38±0.53	1.16±0.55	1.15±0.51	0.0050
Chlorhexidine 0.2% (n = 20)	1.70±0.58	1.29±0.58	1.10±0.52	0.99±0.52	0.0001
Metronidazole+chlorhexidine (n = 20)	1.62±0.60	1.45±0.59	0.98±0.60	0.85±0.52	0.0001
p-value ***	0.703	0.791	0.02	0.001	

*PI: periodontitis index, **Data in rows were analyzed by repeated measure within group test, ***Data in columns were analyzed by one-way ANOVA, post hoc tukey test

Table 3: Oral hygiene condition: percentage of qualifying sites at baseline and various intervals of re-examinations showing visible plaque in four treatment groups of study (n = 20)

	Time			
Groups	Week 0	Week 2	Week 3	Week 4
Placebo	40.50	35.00	35.25	35.50
MTZ	38.25	34.50	29.00	28.75
CHX	42.50	32.25	27.50	24.75
MTZ+CHX	40.50	36.25	24.50	21.25

Table 4: Oral hygiene condition: percentage of qualifying sites at baseline and various intervals of re-examinations showing gingival index in four treatment groups of study (n = 20)

	Time			
Groups	Week 0	Week 2	Week 3	Week 4
Placebo	46.8	41.8	38.3	36.3
MTZ	43.5	37.5	30.3	26.3
CHX	45.5	34.5	31.5	27.3
MTZ+CHX	43.8	32.3	25.0	20.0

Table 5: Percent of qualifying sites at baseline showing BoP at the various examination intervals of re-examinations showing gingival index in four treatment groups of study (n = 20)

	Time			
Groups	Week 0	Week 2	Week 3	Week 4
Placebo	44.0	36.0	32.0	30.0
MTZ	37.0	31.3	22.8	19.0
CHX	42.0	27.3	22.8	20.3
MTZ+CHX	36.8	24.3	18.0	14.3

Table 6: Mean±SD of probing pocket depth (mm) at baseline and changes at 2, 3 and 4 weeks compared to baseline of administration four different toothpastes

	Time of evaluation				
Groups	PPD 0	PPD 2	PPD 3	PPD 4	p-value*
Placebo (control, n = 20)	2.840±0.83	2.740±0.76	2.660±0.73	2.630±0.70	0.001
Metronidazole 1% (n = 20)	2.820±0.83	2.730±0.78	2.660±0.76	2.610±0.73	0.001
Chlorhexidine 0.2% (n = 20)	2.880±0.82	2.780±0.74	2.730±0.70	2.670±0.64	0.001
Metronidazole+chlorhexidine (n = 20)	2.960±0.75	2.860±0.68	2.800±0.63	2.690±0.61	0.001
p-value **	0.783	0.828	0.709	0.927	-

Table 7: Percent difference to baseline of sites showing >1 mm reduction in clinical attachment level (CAL) at various re-examination intervals

	Time of evaluation		
Groups	CAL difference week 2	CAL difference week 3	CAL difference week 4
Placebo (control, n = 20)	7	12	18
Metronidazole 1% (n = 20)	20	14	19
Chlorhexidine 0.2% (n = 20)	3	6	18
Metronidazole+chlorhexidine (n = 20)	9	22	31

Metronidazole versus chlorhexidine: p = 0.016, Metronidazole versus metronidazole+chlorhexidine: p = 0.017

more reduction than other treatment group. Although, there are no significant differences between group analysis, all treatment groups showed a significant decrease of PPD ($p<0.001$) in intra-group analysis at the end of week 4 (Table 6).

Clinical Attachment Level (CAL): Alteration in CAL was recorded at 2, 3 and 4 weeks re-examination for all treatment groups. And there was no significant differences in inter-group analysis. Except for MTZ, intra-group analysis had given significant difference for all other treatment groups at the end of week 4 ($p<0.001$). Table 7 shows the difference of data from the baseline of sites showing >1 mm reduction in clinical attachment level at various re-examination intervals.

During this study nobody reported any side effects such as staining.

DISCUSSION

Up to now local delivery of antimicrobials has not been used widely in maintenance patients. This randomized, double blinded, clinical study examined the effect of various tooth-brushing regimens on the anti-plaque and antimicrobial efficacy of CHX and MTZ alone and combined. The patients recruited to present study were on a maintenance program and had a history of moderate-advanced chronic periodontitis, the SRP treatment phase had been completed at least 1 week prior to baseline. Furthermore, none of the selected target sites had demonstrated any significant reduction in PPD following the non-surgical treatment. After tooth-brushing and providing anti-plaque benefits all data were compared together. An interesting advantage of this vehicle was its compatibility with daily activities.

This study demonstrated that the combined CHX and MTZ toothpaste provides significant anti-plaque benefits when used as an adjunction to SRP and routine oral hygiene programs. Several combined therapies for treatment of periodontal infections have been successfully applied by Greenstein (1992), Grisi *et al.* (2002), Levy *et al.* (2002) and Lindhe *et al.* (1983). In the present study, subjects who brushed with combined toothpaste, showed better overall reduction in the clinical parameters (such as PI, GI and BoP) than CHX or MTZ toothpaste alone. Although the differences of these

clinical parameters between groups were statistically significant, those differences in PPD and CAL were relatively small clinically. The use of CHX as an effective anti-plaque agent has been established (Cosyn and Verelst, 2006).

When CHX containing toothpaste has been brushed three times daily for a month, the tooth brushing led to a significant reduction in the mean PI and GI as well as in BoP up to 30 days after starting therapy but not in PPD and CAL. These results were in accordance with other studies (Colombo *et al*., 2005; Faveri *et al*., 2006; Kaldahl *et al*., 1993; Morrison *et al*., 1980).

The anti-plaque effect of CHX was predominant and this effect was greater at the end of week 4. The efficacy of CHX alone is dose-related (Jenkins *et al*., 1994). This effect appeared similar to the effect of a 0.2% CHX rinse for 1 or 2 times per day (Ainamo *et al*., 1990). These results are in agreement with other studies that confirm the efficiency of CHX alone in controlling supragingival plaque formation (Addy and Moran, 1983; Eaton *et al*., 1997; Faveri *et al*., 2006; Sekino *et al*., 2003; Ziz-Gandour and Newman, 1986).

In addition, daily use of MTZ containing toothpaste, led to a significant reduction in PI similar to CHX, but this reduction is less than CHX+MTZ combination and/or CHX alone as gold standard of anti-plaque chemical (Eaton *et al*., 1997; Loesche, 1979; Sekino *et al*., 2003).

Anti-plaque survey revealed that after 4 weeks subjects who received combined therapy (CHX plus MTZ toothpaste) showed greater improvement in PI than those who received CHX or MTZ toothpaste formulation alone. This result was seen for GI and BoP. Although, reduction in PPD and CAL in all study groups were seen at the end of 30 days, but the differences compared with the baseline and placebo group were not statistical significant. The MTZ result are in consistent with findings of Sculean *et al*. (2001). On the other hand, these data are not in agreement with previous studies that showed pocket depths tend to reduce after therapy (Cosyn and Verelst, 2006; Faveri *et al*., 2006; Saxen and Asikainen, 1993; Sigusch *et al*., 2001). Subjects from the combined therapy exhibited greater reduction in percentage of sites with plaque. The results show that CHX and MTZ have an effective anti-plaque role in adjunction with SRP. This effect may be due to an additive or synergistic effect of combined formulation. On the other hand, drug delivery by brushing with a toothpaste containing antimicrobials, can exert an effective anti-plaque effect on periodontitis. Application of toothpaste form may reduce the risk of CHX-induced staining, as well.

Other formulations of anti-plaque antiseptic CHX are mouthwash (Aziz-Gandour and Newman, 1986; Southern *et al*., 2006), collagen gel (Vinholis *et al*., 2001), local chip (Grisi *et al*., 2002), spray (Bozkurt *et al*., 2004) and chewing gum (Cosyn and Verelst, 2006). Some previous multi-centre studies have shown that CHX as a local chip in conjunction with SRP is an effective treatment in the management of patients with chronic periodontitis. The adjunctive treatments provided significantly greater reductions of PPD for 6 to (Jeffcoat *et al*., 1998; Soskolne *et al*., 1997; Stabholz *et al*., 2000; Vinholis *et al*., 2001) 9 months (Jeffcoat *et al*., 1998) post-treatment. Short-term clinical studies in healthy adolescents and adults have shown an excellent plaque growth-inhibiting effect when chewing gum containing CHX was used (Ainamo and Etemadzadeh, 1987; Simons *et al*., 1999). In a more recently study PI analysis of a CHX chewing gum in young orthodontic patients has shown an excellent plaque growth-inhibiting effect (Cosyn and Verelst, 2006). On the other hand, MTZ has been widely used in treatment of some types of periodontal diseases such as aggressive periodontitis and chronic periodontitis which have not responded to conventional treatment (Pahkla *et al*., 2005). It is active against most established periodontal pathogens and is frequently used alone or combined with amoxicillin as an empirical treatment of periodontitis (Elter *et al*., 1997; Slots and Ting, 2002; Winkelhoff *et al*., 1989; Winkel *et al*., 2001). MTZ has been used as systemic (Pahkla *et al*., 2005; Rooney *et al*., 2002; Sigusch *et al*., 2001) and local administrations (Rudhart *et al*., 1998), e.g., gel (Jansson *et al*., 2003; Kinane and Radvar, 1999; Montebugnoli *et al*., 2002), irrigation form (Pavia *et al*., 2004) and MTZ in resin vehicle (Pattnaik *et al*., 2007). The metronidazole 25% gel in a semi-solid suspension was used in maintenance patients in 2 independent trials (Rudhart *et al*., 1998; Stelzel and Flores-de-Jacoby, 1997). Those studies adopted a split-mouth design to compare the repeated application of metronidazole gel (days 0 and 7) against SRP and showed the 2 treatments to be equivalent with reductions in PPD in the order of 1.32-1.6 mm after 175 days. During one study, MTZ in acrylic resin *vs* CHX irrigation were performed to control of chronic periodontitis, findings showed that there were no significant differences between the groups at any time of the evaluations except for BoP which showed MTZ to be superior (Yeung *et al*., 1983). Administration of various drug delivery systems can be helpful adjunctive therapy for treatment of adult periodontitis.

The significant improvement in indices following administration of combined CHX and MTZ can be related to the increase of the antimicrobial effect of combination. As showed in previous study, MTZ and amoxicillin can exert an additive effect in treatment of AP (Zee *et al*., 2006).

In present study we designed a different combination of MTZ and CHX in toothpaste base as vehicle. Based on our knowledge, this formulation may be the first Iranian preparation which has been used for subjects of this study. We thought that application of toothpaste is compatible with daily activities and patients will be convenient with it. The clinical outcome of this study, confirmed our strategy except for PPD and CAL which only showed minor reduction compared with placebo. It may be due to short duration of this investigation. It is suggested to design a study with CHX and MTZ in a long-term program for obtaining greater changes in PPD and CAL. The main advantages of the present study were low risk of systemic side effects and patient compliance.

Although, there are no question about general effectiveness of MTZ and CHX in the treatment of periodontal diseases, problem can be accounted in the treatment of infections caused by strains with increased resistance (Roberts, 2002). In this case high concentrations of the antimiclobials in the infection site are of critical importance. It seems that tooth-brushing by bass technique with CHX and/or MTZ may lead to release of the drugs from the vehicles in a considerable concentration, also some experiments confirmed that orally used MTZ penetrates well into crevicular fluid (Pahkla *et al*., 2005) in saliva. Although saliva doesn't have access through the gingival pocket, it seems that bass brushing may obtain a high concentration of MTZ or CHX in gingival pockets. New study could be designed to assess the drug's concentration after brushing of the toothpastes.

When the main volume of the dental plaque is removed by mechanical actions (e.g., SRP), the chemopreventive agents enhance the clinical and microbiological effects of that procedure by reduction of biofilm remnants. It was assumed that CHX and MTZ toothpastes could have preventive beneficial effect on plaque formation. Subjects who used combined CHX/MTZ toothpaste showed a greater reduction in clinical parameters (PI, GI and BoP) in compared with CHX or MTZ toothpastes alone.

Tooth-brushing of combined CHX/MTZ toothpaste after SRP could be an effective therapy in plaque reduction in subjects with chronic periodontitis.

In conclusion, the results of the present study indicate that frequent use of CHX or MTZ containing toothpastes alone or combined as an adjunction to existing oral hygiene procedures may reduce plaque levels and gingival bleeding tendency. However, this clinical study suggests that the effect will be predominant when used as a combined formulation of CHX and MTZ. This method could be an option of clinically effective treatment for dental professionals and their patients for long-term management of adult periodontitis.

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