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The effect of toothbrushing regimens on the plaque inhibitory properties of an experimental cetylpyridinium chloride mouthrinse

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Abstract

Objective: The objective of this study was to determine the effect of various toothbrushing regimens with a standard fluoride dentifrice on the plaque inhibitory properties of an alcohol-free, high bioavailable 0.07% cetylpyridinium chloride (CPC) mouthrinse.

Materials and Methods: The study was a randomized, single-centre, examiner blind, four-period cross-over study involving 29 healthy subjects. Four treatment regimens were evaluated: (1) Toothbrushing with dentifrice followed by a water rinse (B-W, negative control); (2) Toothbrushing with dentifrice followed by a CPC mouthrinse use (B-CPC); (3) Toothbrushing with dentifrice followed by a water rinse and then a CPC mouthrinse use (B-W-CPC); and (4) Toothbrushing with dentifrice and waiting 60 min. prior to a CPC mouthrinse use (B-60 min.-CPC). Three days before the baseline exam of treatment periods, subjects were instructed to brush only the lingual surfaces of their teeth for up to 60 s twice daily. At baseline, subjects received a plaque exam using the Turesky modification of the Quigley–Hein index (MQH) followed by a polishing on the lingual and buccal surfaces of their teeth. During treatment periods, subjects were asked to brush only the lingual surfaces of their teeth with a standard fluoride dentifrice. Rinsing with 20 ml of the experimental CPC solution was done for 30 s twice daily. The evening before the last day of treatment periods (Day 4), subjects were asked to refrain from any oral hygiene, eating, and drinking after brushing. On Day 4, plaque was scored using the MQH Index. A 10-day wash-out of normal oral hygiene was allowed between each of the four treatment periods. The data were analysed using analysis of covariance for cross-over designs.

Results: Twenty-five to 29 subjects were evaluable at any given visit. With respect to unbrushed buccal and brushed lingual surfaces, all three CPC regimens had highly significantly ($p \leqslant 0.0006$) lower mean plaque scores than the B-W regimen, reductions ranging from 20% to 38% in magnitude. With respect to unbrushed surfaces, there was a significant difference between the B-CPC regimen and the B-60 min.-CPC regimen ($p < 0.01$) in favour of the latter regimen. No other pairwise treatment comparisons were statistically significant for unbrushed sites. Results for brushed surfaces and all sites combined showed that both the B-W-CPC and the B-60 min.-CPC groups reduced mean plaque levels significantly ($p \leqslant 0.013$) more than B-CPC. There were no statistically significant differences between B-W-CPC and B-60 min.-CPC for measurements of brushed, unbrushed, or all sites combined.

Conclusions: Results show that the alcohol-free, 0.07% high bioavailable CPC rinse provides an additive anti-plaque benefit beyond toothbrushing with a standard fluoride dentifrice regardless of the regimen. Of the regimens, a water rinse between toothbrushing and CPC rinsing enhances therapeutic efficacy while fitting into the patient's typical oral hygiene routine.

Key words: cetylpyridinium chloride; clinical trial; mouthrinse; plaque; surfactant; toothpaste

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Dental plaque is the main aetiologic factor of periodontal diseases (Page 1986, Kornman & Loe 1993). The two most prevalent diseases of the periodontium are plaque-induced gingivitis, a reversible condition, and chronic periodontitis, an irreversible condition that can lead to tooth loss. The role of dental bacterial plaque in the development of these diseases has been established in many studies (Theilade et al. 1967, Holt et al. 1988, Breuer & Cosgrove 1989, Madianos et al. 2005).

It is believed that the best approach to manage periodontal diseases is prevention, followed by early detection and treatment. The prevention of periodontal diseases is targeted at the control of dental plaque (Baehni & Takeuchi 2003). Chemical agents with anti-plaque activities have been shown to represent a valuable complement to mechanical plaque control. (Horwitz et al. 2000, Yates et al. 2002, Mankodi et al. 2004, Paraskevas & van der Weijden 2006). Anti-plaque mouthrinses have been shown to reduce or prevent gingivitis when used as the only oral hygiene practice or when used in combination with traditional mechanical plaque removal procedures such as toothbrushing (Ross et al. 1993, Claydon et al. 1996, Allen et al. 1995, Lucas & Lucas 1999, Hu et al. 2003).

Research has demonstrated that cetylpyridinium chloride (CPC) mouthrinses have anti-plaque activity when used alone and in conjunction with toothbrushing (Ciancio et al. 1975, Ashley et al. 1984, Renton-Harper et al. 1996, Mankodi et al. 2005, Stookey et al. 2005, Witt et al. 2005a, b). These studies among others have led the US Food and Drug Administration Dental Plaque Subcommittee to make the following comments regarding the effectiveness of CPC for treating plaque and gingivitis ''It is reasonable to assume that formulations containing (at least) 72%–76% available CPC are active in reducing plaque and gingivitis (Federal Register 2003)''. A report by Sheen et al. (2003), however, indicated that CPC activity can potentially be affected by interactions between the CPC and toothpaste ingredients, primarily anionic surfactant ingredients. The authors further suggested that the anionic detergent sodium lauryl sulphate (SLS), commonly found in most toothpastes, interacts with CPC to largely inactivate the effect of this cationic antiseptic, a concept that was proposed by earlier researchers (Ciancio et al. 1975). Similar interference with activity has been reported for chlorhexidine rinses and it has been suggested that there should be at least a 30 min. interval between toothbrushing and chlorhexidine use (Barkvoll et al. 1989).

In the US, consumers typically rinse their mouths with water in some fashion following toothbrushing to remove the dentifrice slurry from their mouths. It is likely that this regimen may remove the dentifrice excipients, thereby allowing mouthrinses to have the maximum anti-plaque effect. The purpose of this study was to evaluate the effect that three different toothbrushing regimens have on the anti-plaque activity of an alcohol-free, high bioavailable 0.07% CPC rinse (which meets the proposed bioavailability guidelines of the FDA Dental Plaque Subcommittee) *versus* a toothbrushing only regimen.

Materials and Methods

This was a randomized, single-centre, examiner blind, four-period cross-over, restricted brushing, plaque study. Both the research protocol and written informed consent were reviewed and approved by an institutional review board prior to study initiation. In order to be included in the study, each subject had to provide written informed consent prior to participation, be at least 18 years of age, be in general good physical and oral health, have a minimum of 20 scorable natural teeth including at least four molars, agree to refrain from any form of non-specified oral hygiene during the treatment periods, including use of products such as floss, toothpicks for plaque removal, and chewing gum, agree to brush only the lingual surfaces of teeth during treatment periods, and agree to refrain from any oral hygiene, eating, and drinking after brushing after 23:00 hours the evening before plaque measurements on Day 0 and Day 4 of each of the four treatment periods. Subjects were excluded from the study if they had a medical condition requiring premedication, had taken antibiotics within 2 weeks before the first treatment period and anytime during the duration of the study, had a history of allergies or hypersensitivity to mouthrinse products containing CPC, indicate an inability to comply with study visit requirements, present rampant caries, open or untreated caries, severe gingivitis, or advanced periodontitis requiring prompt treatment, or had removable or orthodontic appliances which interfere with obtaining 20 gradable teeth.

Twenty-nine subjects who met the above study criteria were enrolled and received each of the following treatment regimens:

(1) Toothbrushing with 0.76% sodium monofluorophosphate dentifrice containing (by ingredient label) the anionic surfactant detergent SLS, (Colgate® Cavity Protection, Colgate-Palmolive, New York, NY, USA) followed by rinsing twice with 15 ml of water for 10 s (B-W).
(2) Toothbrushing with dentifrice followed by rinsing with 20 ml of 0.07% CPC mouthrinse (Crest® Pro-Health™ Rinse, Procter & Gamble, Cincinnati, OH, USA) for 30 s (B-CPC).
(3) Toothbrushing with dentifrice, followed by rinsing twice with 15 ml of water for 10 s, followed by rinsing with 20 ml of CPC mouthrinse for 30 s (B-W-CPC).
(4) Toothbrushing with dentifrice, waiting 60 min., and then rinsing with 20 ml of CPC mouthrinse for 30 s (B-60 min.-CPC).

The same dentifrice and CPC mouthrinse was used in all four regimens.

Before the start of the study, subjects were given a prophylaxis. Three days prior to the baseline exam of each treatment period (4), subjects were instructed to brush only the lingual surfaces of their teeth for up to 60 s twice daily using half a brush head of dentifrice and an ADA manual reference toothbrush (American Dental Association, Chicago, IL, USA). At baseline, subjects received a plaque exam by a single, experienced examiner who was used throughout the study to grade plaque using the Turesky modification of the Quigley–Hein index (MQH; Table 1) followed by a dental polishing. Subjects were then asked to brush only the lingual surfaces of their teeth with half a brush head amount of the provided dentifrice for the next 4 days following their assigned treatment regimen.

At the baseline visit of Period 1 subjects were randomly assigned in blocks of four to one of the following treatment sequences: A–B–C–D, B–D–A–C, C–A–D–B, D–C–B–A. The randomization schedule was prepared with an encoded computer programme by an individual who had no direct involve-

ment with study participants or the examiner.

At the beginning of each of the four treatment period subjects received a kit box which contained the appropriate test products based on the randomization scheme assigned to them, dosing cups with appropriate dispensing volumes marked on the cups, a digital timer for timing purposes, and a detailed instruction sheet for that particular regimen. At the conclusion of each period, subjects returned their kit box and the test products were evaluated for consumption by visual inspection to insure compliance.

After subjects were graded for plaque, had their teeth polished, and received their appropriate kit boxes they were asked to perform their first regimen in the clinic under supervision to insure all instructions were clearly understood. All subsequent treatments for that period (7) were done by the subjects at home and unsupervised.

Subjects were asked not to eat or drink anything for 30 min. following their treatment regimen. Subjects were also asked to refrain from eating or drinking after brushing the evening prior to any plaque exam. At the final visit of each 4-day treatment period, subjects received a plaque exam. During the 10-day wash-out period between treatment periods, subjects returned to their normal toothbrushing habits using only the provided acclimation products.

Table 1. Turesky modification of Quigley–Hein index

Score	Criteria
0	No plaque/debris
1	Separate flecks of plaque at the cervical margin of the area
2	A thin continuous band of plaque (up to 1 mm) at the cervical margin of the area
3	A band of plaque wider than 1 mm but covering less than one-third of the crown of the area
4	Plaque covering at least one-third but less than two-thirds of the crown of the area
5	Plaque covering two-thirds or more of the crown of the area
8	Non-gradeable site
9	Missing tooth

Table 2. Baseline demographic characteristics

Baseline characteristic	Statistic or category	All subjects ($n = 29$)
Age (years)	Mean (SD)	38.9 (8.69)
	Minimum–Maximum	25–54
Sex	Female	19 (66%)
	Male	10 (34%)
Ethnicity	Asian Indian	1 (3%)
	Asian Oriental	2 (7%)
	Caucasian	26 (90%)

Statistical methods

It was determined that a sample size of 26 evaluable subjects would result in 80% power to detect a 0.25 (approximately 9%) difference in mean plaque on unbrushed surfaces between a CPC treatment and the B-W treatment. Power calculations were based on a cross-over model error variance of 0.09, estimated from previous clinical research, with the significance level set to 5%.

The average baseline and post-treatment plaque scores were calculated for each subject on brushed sites (lingual), unbrushed sites (buccal), and all sites combined. The mean plaque scores for brushed sites, unbrushed sites, and all sites were each modelled with a separate analysis of covariance (ANCOVA) model using the SAS Version 8.2 (SAS Institute, Cary, NC, USA) Mixed procedure. Each of the three models included fixed class variables for Period and Treatment, with baseline plaque as the covariate; Subject was modelled as a random effect. In each of the three ANCOVA models, all pairwise treatment comparisons were performed at the 5% significance level. Evidence of residual treatment effect was assessed by including a class variable for prior treatment in the ANCOVA models, as described by Ratkowski et al. (1993).

Results

Twenty-five to 29 subjects were evaluable at any given visit. Overall four subjects did not complete all four treatment regimens, mainly due to protocol violations (scheduling conflicts). There were no dropouts for cause or any reported side-effects for any treatment regimen. Subjects ranged in age from 25 to 54 years and had an average age of 39 years. Sixty-six per cent of subjects were female. Twenty-six of the 29 subjects were Caucasian, two subjects were Asian Oriental and one was Asian Indian (Table 2).

Efficacy results are summarized in Table 3 and Fig. 1. With respect to brushed and unbrushed surfaces, all treatment regimens with the CPC rinse produced significantly lower plaque scores than the B-W treatment ($p < 0.0001$). The magnitude of these differences ranged from 20% to 38%.

With respect to unbrushed surfaces, waiting 1 h after brushing before rinsing with CPC resulted in a significantly ($p = 0.0061$) reduced mean MQH score relative to brushing immediately followed by rinsing. However, there was no significant difference in mean MQH scores between B-W-CPC and the B-60 min.-CPC. No other pairwise treatment comparisons were statistically significant. Results for brushed surfaces and all sites combined showed that B-W-CPC and B-60 min.-CPC reduced mean plaque levels significantly ($p < 0.05$) more than B-CPC (Table 3, Fig. 1). There were no statistically significant differences between B-W-CPC and B-60 min.-CPC for any measures.

Testing for carryover in any of the three ANCOVA models indicated there was no significant carryover effect ($p > 0.54$). However, there was a significant period effect in the ANCOVA models for brushed sites ($p = 0.04$), unbrushed sites ($p < 0.0001$), and all sites combined ($p = 0.0003$). The nature of the period effect was consistent across all three models in that mean plaque scores were lower in Periods 1 and 4 than in Periods 2 and 3. For example, the adjusted overall mean plaque scores in Periods 1, 2, 3, and 4 were 1.54, 1.74, 1.82, and 1.64, respectively. The cause of the period effect is unknown, but the irregular temporal

Table 3. Efficacy analysis – Turesky modified Quigley–Hein plaque index for brushed, unbrushed, and all sites

Treatment	Adjusted mean (SE)	% Difference *versus* placebo	Treatment comparisons		
			B-CPC	B-W-CPC	B-60 min.-CPC
Unbrushed sites*					
B-W	3.07 (0.081)		<0.0001	<0.0001	<0.0001
B-CPC	2.44 (0.080)	21		0.15	0.006
B-W-CPC	2.29 (0.081)	25			0.17
B-60 min.-CPC	2.15 (0.082)	30			
Brushed sites†					
B-W	1.12 (0.066)		0.0006	<0.0001	<0.0001
B-CPC	0.90 (0.066)	20		0.0039	0.001
B-W-CPC	0.72 (0.066)	36			0.65
B-60 min.-CPC	0.69 (0.067)	38			
All sites‡					
B-W	2.11 (0.056)		<0.0001	<0.0001	<0.0001
B-CPC	1.69 (0.055)	20		0.013	0.0002
B-W-CPC	1.52 (0.056)	28			0.16
B-60 min.-CPC	1.43 (0.056)	32			

*Baseline mean = 3.13.
†Baseline mean = 1.52.
‡Baseline mean = 2.33.
CPC, cetylpyridinium chloride.

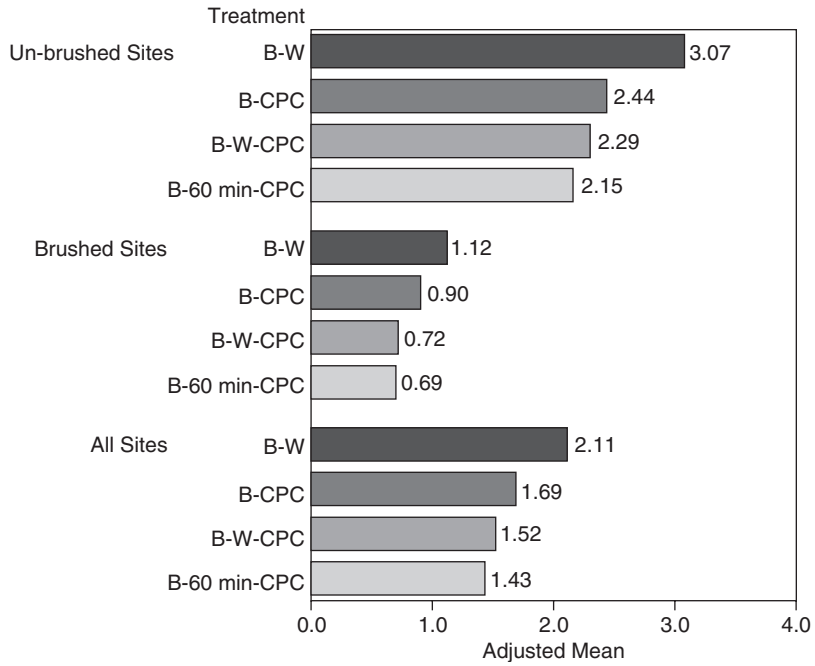

Fig. 1. Treatment comparisons – analysis of covariance adjusted means (Turesky modified Quigley–Hein plaque index for brushed, unbrushed, and all sites).

pattern suggests it was not due to subject behavioural changes that developed over time.

To assess the impact that the period effect had on study results, adjusted treatment means were compared with and without Period in the ANCOVA models. When Period was removed from the models, adjusted treatment mean changes ranged from −0.04% to 1.9%. The minimal impact of removing Period from the model can be attributed to the fact that treatments were well balanced in each treatment period. Though treatment comparison *p*-values increased when Period was dropped from the models, due to inflated error variance estimates, no pairwise treatment comparison changed from statistically significant to statistically insignificant or vice versa at the 5% significance level. In summary, though there was a statistically significant period effect in the ANCOVA models, there was no evidence to indicate that the effect had any practical impact on study results.

Discussion

This randomized, examiner blind, crossover study examined the effect of various toothbrushing regimens on the anti-plaque efficacy of an alcohol-free, 0.07% high bioavailable CPC mouthrinse. The trial specifically evaluated whether a water rinse imposed between toothbrushing and use of a CPC mouthrinse would be sufficient to remove any potentially interfering dentifrice excipients from the mouth and provide anti-plaque benefits similar to those obtained by waiting for 60 min. after toothbrushing as recommended in a previous study using dentifrice slurry with no brushing (Sheen et al. 2003). This study demonstrated that the CPC mouthrinse provides significant anti-plaque benefits when used as an adjunct to various toothbrushing regimens, including a regimen with CPC rinsing immediately following toothbrushing, *versus* toothbrushing alone. Brushing with standard toothpaste and rinsing with water before using the CPC mouthrinse was not statistically different from brushing with toothpaste and waiting 60 min. before using the CPC mouthrinse. Both regimens enhanced the efficacy of the CPC mouthrinse compared with a regimen of CPC rinsing immediately following toothbrushing. A water rinse following toothbrushing regimen is preferred, as it is consistent with labelled usage instructions and typical consumer toothbrushing habits.

Results of this study demonstrate that while CPC activity may be affected by excipients in dentifrice, the high bioavailable, 0.07% CPC mouthrinse tested in this study still exhibited statistically significant anti-plaque benefits even when used immediately after toothbrushing (e.g., no water rinse or waiting period between brushing and rinsing). There are several factors that could explain the difference in results between this study and the report of Sheen and colleagues that recommended a 60 min. waiting period between toothbrushing and CPC rinsing. First, the Sheen study

tested 10 ml of a 0.05% CPC mouthrinse whereas the present study tested 20 ml (consistent with product's usage instructions) of a novel 0.07% high bioavailable, alcohol-free CPC rinse. Thus, the total CPC available to provide therapeutic activity in the Sheen study was lower than the current trial. Second, subjects in the Sheen study rinsed with dentifrice slurry while those in the present trial performed partial toothbrushing, which is closer to typical consumer habits. Delivering dentifrice in a slurry form *versus* via toothbrushing could increase the contact and retention of dentifrice excipients on the soft tissue, increasing the odds of interference with CPC, which is retained on soft tissue. Moreover, Sheen and colleagues did not evaluate the impact of a water rinse between the dentifrice exposure and CPC rinse and therefore this option was not taken into consideration when they made the recommendation to wait an hour post-brushing before CPC use. Some reports have suggested that rinsing with water after toothbrushing may decrease the activity of the fluoride in the dentifrice, but a 3-year prospective clinical trial comparing post-brushing rinsing behaviours among children shows there is no negative impact on the incidence of caries. (Machiulskiene et al. 2002).

In conclusion, the 0.07% high bioavailable, alcohol-free mouthrinse tested in this study was shown to provide significant anti-plaque activity when used: immediately following toothbrushing with a standard fluoride dentifrice; after a water rinse that followed toothbrushing; and after waiting 60 min. following toothbrushing. The recommended regimen to enhance the CPC activity while complementing typical oral hygiene practices is to perform a water rinse between toothbrushing and rinsing with the 0.07% CPC rinse. This is the procedure which was followed in a 6 month study (Mankodi et al. 2005) utilizing this mouthrinse which showed both statistically significant and clinically relevant plaque and gingivitis reductions. The plaque results should not automatically be extrapolated to other CPC mouthrinses, as the concentration and bioavailability of the CPC in other products may not meet proposed regulatory guidelines.

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Clinical Relevance

Scientific rationale for study: Previous research has suggested that one should wait some period of time after brushing one's teeth before using a CPC mouthrinse to avoid its inactivation by dentifrice ingredients.

Principal findings: The present study evaluated the anti-plaque efficacy of a high bioavailability, alcohol-free 0.07% CPC mouthrinse under three different tooth brush/mouthrinsing regimens. All treatment regimens were found to be effective at reducing plaque *versus* the negative control.

Practical implications: This study demonstrates the CPC mouthrinse is an effective approach beyond brushing alone to control plaque under several different oral hygiene regimens.