Contemporary Clinical Trials Communications 8 (2017) 85–89

Contents lists available at ScienceDirect

Contemporary Clinical Trials Communications

journal homepage: www.elsevier.com/locate/conctc

Microbiological and clinical effects of an oral hygiene regimen

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ARTICLE INFO

Keywords:
Dental plaque
Bacteria
Mouthwash
Hygiene
Gingivitis

ABSTRACT

Objective: This study compared the additional effect of rinsing with a fluoride-free and alcohol-free 0.075% cetylpyridinium chloride (CPC) mouthwash to brushing alone on dental plaque, gingival inflammation, and supragingival plaque bacteria.

Methods: Adult subjects [n = 68] completed a washout period prior to baseline evaluations that evaluated gingival inflammation, gingival bleeding, dental plaque, and pocket probing depths along with microbiological analysis of supragingival plaque for bacteria. Subjects were randomized to two treatment groups: brush with fluoride toothpaste and rinse with the CPC mouthwash (test) or brush with fluoride toothpaste only (control), twice daily for the next four weeks. Subjects abstained from oral hygiene for twelve-hours prior to two-week and four-week post-treatment microbiological analysis of supragingival plaque for bacteria. Clinical assessments for gingival inflammation, gingival bleeding, dental plaque, and pocket probing depths were conducted at the four-week post-treatment visit.

Results: Compared to baseline, bacteria of dental plaque in the test group were reduced by 61.1% and 83.0% at the two-week and four-week evaluations, respectively ($p < 0.05$). Compared to baseline, bacteria of supragingival plaque in the control group were reduced by 2.3% at either post-treatment evaluations ($p < 0.05$). Additionally, dental plaque bacteria in the test was 69.8% and 86.8% lower than the control at the two-week and four-week evaluations ($p < 0.05$), respectively. After four-weeks, the test group showed 14.3% less gingivitis, 11.2% less dental plaque, 7.5% less gingival bleeding compared to the control group ($p < 0.05$).

Conclusions: Oral hygiene comprising toothbrushing and rinsing with a mouthwash containing 0.075% cetylpyridinium chloride demonstrated greater reductions of dental plaque bacteria, improving gingival health, and eliminating supragingival plaque than toothbrushing alone.

Clinical relevance

Scientific rationale for study: Poor oral hygiene resulting in microbial accumulations of dental plaque has been associated with common conditions such as gingivitis. This study utilized a unique study design to examine improvements in oral hygiene provided by a regimen comprising toothbrushing and rinsing with a cetylpyridinium chloride mouthrinse than toothbrushing alone. Study evaluated effects on oral bacteria and on supragingival plaque and gingivitis over the study duration.

Principal findings: A regimen comprising toothbrushing and rinsing with a cetylpyridinium chloride mouthrinse demonstrated significantly greater reductions in oral bacteria and clinical outcomes than toothbrushing alone. Additionally, the regimen demonstrated progressive improvements in evaluated outcomes over the study period.

Practical implications: Oral hygiene comprising toothbrushing and rinsing with a mouthwash containing 0.075% cetylpyridinium chloride demonstrated greater reductions of dental plaque bacteria, improving gingival health, and eliminating supragingival plaque than toothbrushing alone.

1. Introduction

The most common oral health regimen in Western nations is brushing with fluoride-containing toothpaste to remove dental plaque and prevent dental caries [1–5]. Many people complement this practice with additional methods of hygiene [1–3]. Cleaning interproximally with floss or a brush, removing detritus from the tongue with a brush or a scraper, using an oral irrigator, and/or rinsing with mouthwash can all help reduce plaque, manage halitosis, and avoid tooth decay [1–3].

While the efficacy of certain aspects of oral hygiene, such as toothbrushing, has been well established, there are less data on the virtue of other facets of dental self-care. For example, there is strong evidence to show that daily brushing with a dentifrice containing

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http://dx.doi.org/10.1016/j.conctc.2017.08.010
Received 3 February 2017; Received in revised form 26 July 2017; Accepted 15 August 2017
Available online 18 August 2017

fluoride prevents more dental caries than using a non-fluoride toothpaste [5]. Studies also affirm that toothbrushing combined with flossing may reduce dental plaque, with concomitant reductions in gingivitis, more than brushing alone [1–3,6]. There is also data to support the idea that children who use a fluoride mouthrinse in addition to brushing their teeth with a fluoride toothpaste have fewer dental caries than children who use fluoride toothpaste alone [7,8]. In addition, a review of five previously conducted clinical trials found that subjects who only brushed their teeth showed less improvement in halitosis than those who brushed in addition to using mouthrinses containing chlorhexidine, cetylpyridinium chloride, or chlorine dioxide and zinc [9].

In the present study, we hypothesized that patients who brushed with fluoride-containing toothpaste in addition to rinsing with a fluoride- and alcohol-free mouthwash containing 0.075% cetylpyridinium chloride (CPC) would show greater reduction in dental plaque organisms than those who used fluoride toothpaste alone.

2. Materials and Methods

This was a double-blind, two-treatment, parallel design, randomized controlled clinical trial conducted at a single site. At the screening appointment conducted at the School of Dental Medicine, University of Buffalo, male and female volunteers between 18 and 70 years of age completed an informed consent form, a health screening form, and a demographic questionnaire. They were then evaluated by a dentist for oral soft and hard tissue health and underwent whole-mouth evaluations assessing six sites per tooth for gingival inflammation (Loe-Silness Index) [10], gingival bleeding, [11] and dental plaque [12]. Subjects who met the following criteria were eligible for participation in the study: (1) good general health, (2) ability to read, understand, and sign the informed consent form; (3) willingness to comply with study procedures and sampling schedules, (4) at least 20 uncrowned permanent natural teeth, (5) gingival index ≥ 1.0[10], and (6) plaque index ≥ 1.5[12]. Subjects were excluded from the study if they (1) had a history of significant adverse effects caused by oral hygiene products, (2) had allergies to personal care products or their ingredients, (3) had gross dental caries or extensively restored facial or lingual tooth surfaces, (4) had fixed or removable orthodontic appliances, (5) had removable partial dentures, (6) had a history of, or current, diabetes mellitus, renal disease, heart disease, alcoholism, recreational drug use or other serious medical conditions or transmissible infectious diseases (such as hepatitis or AIDS); (7) required antibiotics prior to dental treatment, (8) used antibiotics, anti-inflammatory drugs, or anticoagulants in the prior month; (9) had significant oral pathology (including, but not limited to, gingival enlargement, severe gingivitis, moderate to severe periodontitis including $\geq$ one periodontal pocket $>$ 5 mm); (10) had participated in a clinical study involving oral care products in the prior month; (11) reported currently being pregnant or breast-feeding, or (12) had lip or tongue piercings.

The details of the study were explained to each eligible subject, who were given the opportunity to ask for any needed clarification. They acknowledged their consent and their willingness to comply with study procedures and sampling schedules by signing the informed consent form. The clinical protocol and consent forms were reviewed and approved by the University at Buffalo Health Sciences Institutional Review Board with the study conducted at the School of Dental Medicine, University at Buffalo.

Subjects were instructed to refrain from oral hygiene for 12 h and from food, drink or smoking for at least 4 h before the baseline, two-week and four-week examinations. At the baseline and four-week examinations, the subjects underwent whole-mouth evaluations at six sites for clinical parameters. Clinical evaluations included assessments for gingival inflammation (Loe-Silness Index) [10], gingival bleeding, [11] and dental plaque [12] and full-mouth pocket probing depths using a University of Michigan probe [13]. Supragingival dental plaque was collected at the baseline, two-week, and four-week examinations for microbiological analysis of bacteria. At each examination, plaque samples for microbiological analyses were randomly collected from either the upper right or left quadrants.

After the baseline examination and dental plaque sampling conducted by a dental professional, the subjects were randomly assigned to test or control groups using a computer-generated assignment sequence by a study co-ordinator. Subjects assigned to the test group were instructed to brush twice daily (morning and evening) with a commercially available regular fluoride toothpaste (Colgate Dental Cream, Colgate Great Regular Flavor, Colgate-Palmolive Company New York, NY) and soft-bristled toothbrush (Colgate Extra Clean, Colgate-Palmolive Company New York, NY). After brushing, subjects were instructed to rinse for 30 s with 20 ml of a fluoride- and alcohol-free mouthwash containing 0.075% CPC (Colgate Total, Colgate-Palmolive Company New York, NY). Subjects assigned to the control group were instructed to brush twice daily (morning and evening) with a commercially available regular fluoride toothpaste (Colgate Dental Cream, Colgate Great Regular Flavor, Colgate-Palmolive Company New York, NY). All products were overwrapped, coded and supplied by Colgate-Palmolive Company, New York, NY with subjects and dental examiners blinded to treatment assignment. Subject recruitment commenced in March 2013 and the study completed in July 2013.

Supragingival dental plaque samples obtained at the baseline, two-week and four-week examinations were dispersed by sonication and then serially diluted by tenfold in phosphate-buffered saline. Undiluted samples and sample dilutions 10^1 to 10^4 were distributed in duplicate (Spiral Systems Autoplate 4000 Spiral Plater) on enriched trypticase soy agar containing 5% sheep blood (ETSA). The inoculated media were incubated at 37 °C for 5–7 days under anaerobic conditions. Viable counts for each sample was recorded as mean colony-forming units (CFU)/ml from duplicate cultures from dilutions demonstrating at least 20 colony-forming units. Results from viable counts were log transformed ($\log_{10}$) for analysis.

3. Statistical analysis

Sample size calculations were based on unpublished historical data that a sample size of 30 subjects would detect a difference of 0.6 Log CFU/ml in viable plaque bacteria between treatments at 80% probability assuming a standard deviation of 0.7 Log CFU/ml for bacteria (unpublished data). Viable counts from the bacterial cultures were calculated as the mean colony-forming units (CFU)/ml of duplicate cultures from dilutions demonstrating at least 20 colony-forming units. The viable counts were log transformed ($\log_{10}$) for analysis. Mean scores were computed for gingival index, bleeding index and plaque index scores at the baseline and four-week whole mouth examinations. Demographic results between the two treatment groups were compared by a chi-square analysis. Clinical and microbiological data were analyzed using analysis of variance (ANOVA), paired t-tests, and analysis of covariance (ANCOVA). All statistical tests of hypotheses were two-sided and employed a level of significance of $\alpha = 0.05$. Analyses were conducted with Minitab (Minitab Inc., State College, PA).

4. Results

The demographic data for the study population screened and enrolled are presented as a CONSORT diagram (Fig. 1). There were 34 subjects in both the test and control groups (Table 1) who completed the entire study. Statistical analyses indicate no significant ($p > 0.05$) differences between test or control groups at baseline for any of the clinical or microbiological assessments (Table 1). There were no observed or reported adverse events on the oral soft or hard tissues observed by the clinical examiner or reported by the subjects during the study.

Table 2 presents a summary of the mean number of cultivable oral bacteria (log CFU/ml) collected twelve (12) hours after two and four