Comparison of efficacy of an arginine-calcium carbonate-MFP toothpaste to a calcium carbonate-MFP toothpaste in controlling supragingival calculus formation and gingivitis: A 6-month clinical study

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ABSTRACT: Purpose: To investigate whether the long-term use (6 months) of an arginine-calcium carbonate-MFP toothpaste would affect calculus formation and/or gingivitis when compared to a calcium carbonate-MFP toothpaste. **Methods:** This was a double-blind clinical study. Eligible adult subjects (120) entered a 2-month pre-test phase of the study. After receiving an evaluation of oral tissue and a dental prophylaxis, the subjects were provided with a regular fluoride toothpaste, a soft-bristled adult toothbrush with instructions to brush their teeth for 1-minute twice daily (morning and evening) for 2 months. The subjects were then examined for baseline calculus using the Volpe-Manhold Calculus Index (VMI) and gingivitis using the Löe-Silness Gingival Index (GI), along with an oral tissue examination. Qualifying subjects were randomized to two treatment groups: (1) Colgate Sensitive Pro-Relief toothpaste containing 8.0% arginine, 1450 ppm MFP and calcium carbonate (Test group), or (2) Colgate Cavity Protection toothpaste containing 1450 ppm MFP and calcium carbonate (Control group). Subjects were stratified by the VMI score and gender. After a dental prophylaxis (VMI=0), the subjects entered a 6-month test phase. Each received the assigned toothpaste and a soft-bristled adult toothbrush for home use with instructions of brushing teeth for 1 minute twice daily (morning and evening). The examinations of VMI, Löe-Silness GI and oral tissues were conducted after 3 and 6 months. Prior to each study visit, subjects refrained from brushing their teeth as well as eating and drinking for 4 hours. **Results:** 99 subjects complied with the study protocol and completed the 6-month test phase. No within-treatment comparison was performed for the VMI because it was brought down to zero after the prophylaxis at the baseline of the test phase. For the Löe-Silness GI, subjects of the Test group exhibited a significant difference from baseline at the 3- and 6-month examinations. The 3-month Löe-Silness GI of the Control group was significantly different from that of the baseline; however, its 6-month Löe-Silness GI was not statistically significantly different from the baseline values. After 3 and 6 months, there were no significant differences between the Test and Control groups with respect to the mean VMI scores; there were no statistically significant differences between the two groups with respect to the Löe-Silness GI results after 3 and 6 months of product use. (*Am J Dent* 2012;25:21-25).

CLINICAL SIGNIFICANCE: The use of Colgate Sensitive Pro-Relief toothpaste containing 8.0% arginine, 1450 ppm MFP and calcium carbonate for 3 and 6 months did not increase risks of calculus formation and gingivitis.

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Introduction

Dentin hypersensitivity is a common complaint of patients seeking dental treatment.[1] As defined by Ajcharanukul *et al*,[2] dentin hypersensitivity is a short, sharp pain arising from exposed dentin in response to stimuli typically thermal, evaporative, tactile, osmotic, or chemical, and which cannot be ascribed to any other form of dental defect or pathology. A number of etiological factors causing tooth sensitivity has been identified.[3] A key characteristic common to these causes of tooth sensitivity is the exposed dentin, such as gingival recession and worn enamel due to acid erosion and/or mechanical abrasion, through which the stimuli activate the pulpal nerves. Tooth bleaching using peroxide-based materials is also known to cause tooth sensitivity, although in most cases it is mild to moderate, transient, and dissipates spontaneously without specific treatment.[4]

A variety of chemicals, products and measures has been used by professionals in-office or by patients at-home to combat the tooth sensitivity. Recently a novel technology has been introduced to control tooth sensitivity. The formulation contains 8.0% arginine, which is an amino acid found in saliva.[5] The mechanism of this new desensitizing formulation mimics saliva's natural process of plugging and sealing open dentin tubules. The dentin sealing plugs so formed are composed of arginine, calcium, phosphate and carbonate. Their strength is adequate to withstand normal pulpal pressures and acid challenge, effectively reducing the dentin fluid flow and consequently the sensation of tooth sensitivity.[6-9]

It is theoretically possible that arginine delivered to the mouth during product use could subsequently break down to form basic conditions which may promote calculus formation. To probe this hypothesis, the objectives of this two-cell, double-blind clinical study were: (1) to investigate whether the long-term use of Colgate Sensitive Pro-Relief[a] toothpaste containing 8.0% arginine, 1450 ppm MFP and calcium carbonate (Test toothpaste) resulted in an increase in supragingival calculus formation, as compared to Colgate Cavity Protection[a] toothpaste containing 1450 ppm MFP and calcium carbonate (without arginine) (Control toothpaste) after twice daily use of the products for 6 months; and, (2) to assess if the long-term use of the Test toothpaste resulted in a change in gingivitis status as compared to the Control toothpaste.

Materials and Methods

Prior to the initiation of the study, the protocol and the letter of informed consent were reviewed and approved by the Institutional Review Board (IRB) of Loma Linda University. Among 286 subjects screened, 120 were eligible and participated in this randomized, double-blind, two-treatment, longitudinal clinical study. The participants were recruited from local communities through advertisements in newspapers; eligible subjects had to sign the informed consent form and meet the following criteria:

1. Be between 18 and 70 years of age, and in good general health;
2. Have six scoreable mandibular anterior teeth free of large restorations or dental prosthetic crowns;
3. Have a pre-test phase Volpe-Manhold calculus index (VMI) score ≥ 7.0; and
4. Be available for the 32-week duration of the study (8-week pre-test phase and 24-week test phase).

A prospective subject was excluded if he or she had any of the following conditions: orthodontic appliances, ≥ one mandibular anterior tooth with a prosthetic crown or veneer, tumors of the soft or hard oral tissues, moderate or advanced periodontal disease, ≥ five carious lesions requiring immediate care, or a history of allergies to dentifrice or personal care products. Additionally, subjects also excluded were pregnant or lactating women, individuals participating in any other clinical study, or unable to refrain from eating and drinking for at least 4 hours prior to scheduled study visits or using antibiotics or steroids any time during the 1 month prior to the study.

Prior to the 6-month test phase, the subjects participated in a 2-month pre-test phase. Prospective subjects reported to the clinical facility having refrained from brushing their teeth, eating and drinking for 4 hours prior to their examination. Subjects signed an informed consent and were evaluated by a dental examiner for inclusion/exclusion criteria. Examinations for entering the pre-test phase included oral soft and hard tissues, supragingival calculus using the VMI scoring method[10-15] and gingivitis using the Löe-Silness gingival index (GI) scoring method.[16]

A single examiner performed the oral tissue and VMI examination for all subjects throughout the study, while the GI of all subjects was examined by another investigator. Both examiners were experienced and calibrated, each with more than 10 years of clinical research and publications involving the use of the clinical parameters they performed in the present study. The oral tissue examination was conducted by visual observation of oral cavity and peri-oral area using a dental light and dental mirror, including soft and hard palate, gingival and buccal mucosa, mucogingival fold areas, tongue, sublingual and submandibular areas, salivary glands, and the tonsilar and pharyngeal areas. For the VMI, the method measures supragingival calculus in three planes (mesial, mid and distal) with a periodontal probe graduated in millimeters on the lingual surfaces of the six mandibular anterior teeth; the resulting 18 site-wise measurements are added to obtain an overall score for each subject. The Löe-Silness GI was determined by evaluating the gingival health of all natural teeth, excluding third molars, according to the following criteria:

0 = Absence of inflammation;
1 = Mild inflammation: slight change in color and texture;
2 = Moderate inflammation: moderate redness, edema, glazing, hypertrophy; bleeding on probing;
3 = Severe inflammation: marked redness and hypertrophy, a tendency to spontaneous bleeding (elicited by air syringe) and/or ulceration.

Qualified subjects received a complete dental prophylaxis and were then provided with a placebo dentifrice (Colgate Cavity Protection) and an adult soft-bristled toothbrush, with instructions to brush their teeth twice daily (morning and evening) as they normally did, each for 1 minute, for 2 months.

After completing the pre-test phase, subjects returned to the clinical facility for the same examinations of oral tissues, VMI and Löe-Silness GI by the same examiners. The subjects were then stratified according to their baseline VMI score and gender and were randomly assigned within strata to one of the following two groups:

Test group: Colgate Sensitive Pro-Relief toothpaste;
Control group: Colgate Cavity Protection toothpaste.

Subjects then entered the 6-month test phase. After a complete dental prophylaxis to achieve a baseline VMI score of zero for the start of the test phase, the subjects were provided with a soft-bristled adult toothbrush and their assigned toothpaste, which was overwrapped with white labels to maintain the double-blind study design. Subjects were instructed to brush their teeth with their assigned toothpaste and toothbrush as they normally did for 1 minute twice daily (morning and evening) and to refrain from routine dental treatment during the study. After 3 and 6 months of product use, all subjects were again evaluated for the oral tissues, VMI and Löe-Silness GI by the same examiners.

For each clinical visit, the subjects were asked to refrain from brushing their teeth, eating and drinking for 4 hours prior to the scheduled examinations. Adverse events were obtained from an interview with the subjects and from the oral examination at each visit.

The clinical data were compiled and analyzed by a biostatistician. The data analyses were performed separately for the VMI and Löe-Silness GI assessments. Comparisons of the treatment groups with respect to gender were performed using a chi-square analysis and an independent t-test for age. Comparisons of the treatment groups with respect to the after baseline examination VMI and Löe-Silness GI scores were performed using an Independent t-test. Within-treatment comparisons of the baseline versus follow-up Löe-Silness GI scores were performed using the paired t-tests. Comparisons of the treatment groups with respect to VMI and Löe-Silness GI scores at the follow-up examinations were performed using ANCOVA. All statistical tests of hypotheses were two-sided, and employed a level of significance of $\alpha = 0.05$.

Results

Ninety-nine subjects complied with the protocol and completed the 6-month test phase of the study. Among the 21 subjects who did not complete the study, two (one in each

Table 1. Age and gender distribution of the subjects who completed the 6-month test phase.

	Number of subjects			Age	
Group	Male	Female	Total	Mean*	Range
Test toothpaste	21	34	55	42.5	18 - 69
Control toothpaste	16	28	44	41.2	18 - 69

* There was no significant difference in either gender or age between the two groups.

Table 2. Baseline Volpe-Manhold calculus and Löe-Silness gingival index scores for subjects who completed the 6-month test phase.

Group	N	VMI*	Löe-Silness GI*
Test toothpaste	55	19.17 ± 22.76	1.24 ± 0.21
Control toothpaste	44	21.30 ± 20.64	1.22 ± 0.15

* Mean ± S.D. After baseline examination, a dental prophylaxis was conducted so that the VMI scores became zero. Values within lines are not significantly different.

Table 3. Three- and 6-month Volpe-Manhold calculus index scores for subjects who completed the 6-month test phase.

		3-month VMI	Between treatment comparison		6-month VMI	Between treatment comparison	
Group	N	Mean ± S.D.	% difference [1]	Significance [2]	Mean ± S.D.	% difference [3]	Significance [2]
Test toothpaste	55	18.48 ± 20.55	-7.3%	NS	18.95 ± 23.29	-2.7%	NS
Control toothpaste	44	17.22 ± 16.51			18.45 ± 17.11		

[1] Difference between the 3-month means of the two groups. A positive value indicates less tartar formation for the Test toothpaste relative to the Control toothpaste.
[2] Significance of ANCOVA comparison of baseline-adjusted means.
[3] Difference between the 6-month means of the two groups. A positive value indicates less tartar formation for the Test toothpaste relative to the Control toothpaste.

Table 4. Three-month Löe-Silness gingival index scores for subjects who completed the 6-month test phase.

		3-month GI	Within treatment [1]		Between-treatment [2]	
Group	N	Mean ± S.D.	% change	Significance	% difference	Significance
Test toothpaste	55	1.13 ± 0.15	8.9%	P< 0.05	1.7%	NS
Control toothpaste	44	1.15 ± 0.11	5.7%	P< 0.05		

[1] Percent change exhibited by the 3-month mean relative to the baseline mean. A positive value indicates a reduction in gingival index scores at the 3-month examination. Significance of paired t-test comparing the baseline and 3-month examinations.
[2] Difference between the 3-month means of the two groups. A positive value indicates a reduction in gingival index scores for the Test toothpaste relative to the Control toothpaste. Significance of ANCOVA comparison of baseline-adjusted means.

Table 5. Six-month Löe-Silness gingival index scores for subjects who completed the 6-month test phase.

		6-month GI	Within treatment [1]		Between-treatment [2]	
Group	N	Mean ± S.D.	% change	Significance	% difference	Significance
Test toothpaste	55	1.18 ± 0.20	4.8%	P< 0.05	0.8%	NS
Control toothpaste	44	1.19 ± 0.14	2.5%	NS		

[1] Percent change exhibited by the 6-month mean relative to the baseline mean. A positive value indicates a reduction in gingival index scores at the 3-month examination. Significance of paired t-test comparing the baseline and 6-month examinations.
[2] Difference between the 6-month means of the two groups. A positive value indicates a reduction in gingival index scores for the Test toothpaste relative to the Control toothpaste. Significance of ANCOVA comparison of baseline-adjusted means.

group) did not return for the scheduled visit, and 19 (four in the Test group and 15 in the Control group) requested to discontinue their participation due to lack of transportation, moving out of state, schedule conflicts, pregnancy, or non-specified personal reasons; none was related to the use of the assigned toothpaste or disqualified during the study. Subjects received free oral examinations, toothpaste, toothbrush and a small monetary gift for their participation in the study.

Table 1 summarizes the gender distribution, average age and age range of the study population, which were comparable between the two groups. Throughout the study, there were no adverse events of the oral soft or hard tissues of the oral cavity detected during the clinical examinations or reported by the subjects when questioned at each visit.

The VMI and Löe-Silness GI data measured at the baseline (prior to the dental prophylaxis) for the subjects who completed the 6-month test phase of the study are presented in Table 2. For the VMI, the baseline mean scores were 19.17 and 21.30 for the Test and Control groups, respectively. The baseline Löe-Silness GI scores were 1.24 for the Test group and 1.22 for the Control group. There was no statistically significant difference (P> 0.05) between the two groups with respect to either the baseline VMI or Löe-Silness GI scores.

Tables 3 and 4 provide the 3-month results of the calculus and gingivitis examinations. For the VMI data, no comparisons to the baseline were made for the within-treatment effect because it was zero for both groups after the dental prophylaxis at the baseline. For the between-treatment effect, the analysis found no statistically significant difference (P> 0.05) in mean VMI scores between the Test and Control groups (the percentage difference between the two groups was 7.3%) after 3 months of product use (Table 3). As shown in Table 4, the mean 3-month Löe-Silness GI scores were 1.13 and 1.15 for the Test and Control groups, respectively. The within-treatment

percent changes from baseline were 8.9% for the Test group and 5.7% for the Control group, both of which were statistically significant (P< 0.05). However, there was no statistically significant difference (P>0.05) on the between-treatment effect of the two groups using the Löe-Silness GI scores (1.7%) (Table 4).

The 6-month data are presented in Tables 3 and 5. Again, no comparisons of the VMI data to the baseline were made because it was zero for both groups after the dental prophylaxis at the baseline. The analysis of the between-treatment effect showed no statistically significant difference (P> 0.05) in mean VMI scores between the Test and Control groups (the percentage difference between the two groups was 2.7%) after 6 months of product use (Table 3). The mean Löe-Silness GI scores at 6 months were 1.18 for the Test group and 1.19 for the Control group. The change from baseline for the Test group was 4.8%, which was statistically significant (P<0.05); however, the 2.5% change for the Control group was not statistically significant (Table 5). When the two groups were compared, the difference in the Löe-Silness GI scores of 0.8% was not statistically significant after 6 months of product use (P> 0.05).

Discussion

Dental calculus is formed through the process of calcification of dental plaque, with hydroxyapatite, whitlockite and octocalcium phosphate commonly present in mature calculus.[9,17] The rate of calculus formation varies from person to person and can be increased by such factors as elevated salivary pH, concentration of salivary calcium, urea, bacterial proteins or lipids. Colgate Sensitive Pro-Relief toothpaste contains 8.0% arginine as the active ingredient for combating tooth sensitivity. It is possible that arginine delivered to the mouth during product use could break down to form ammonia, which may increase salivary pH and, thus, may affect calculus formation. The present study, which was a two-cell, double-blind, 6-month clinical investigation, was conducted to determine whether this phenomenon occurred in the clinical setting.

The results demonstrated that regular twice daily brushing with Colgate Sensitive Pro-Relief toothpaste for 6 months did not increase the formation of supragingival calculus as compared to a fluoride only control toothpaste. As shown in Table 2, the average VMI at the baseline of the test phase was around 20, which was the net accumulation in 2 months between the dental prophylaxis to achieve a zero VMI at the beginning of the pre-test phase and the baseline of test phase. As the subjects again received the dental prophylaxis to achieve a zero VMI at the beginning of the test phase, the average calculus accumulated during the first 3 months of the test phase was around 18 VMI (Table 3), indicating a study population of moderate and consistent calculus formation. There was only a slight increase in the VMI for both groups (0.47 and 1.23 for the Test and Control groups, respectively) during the second 3 months of the test phase (Table 5), showing that the calculus accumulation mostly occurred during the first 2 to 3 months and then stabilized.

Regular brushing with either the Test or Control toothpaste provided a beneficial effect of reducing gingivitis consistent with the motivational effects of participating in the clinical study. Such beneficial effect appeared more consistent for the Test toothpaste. As shown in Tables 4 and 5, after 3 and 6 months of product use, subjects in the Test group exhibited a significantly (P< 0.05) reduced gingivitis score compared to the baseline value (8.9% and 4.8% respectively). However, during the same periods, subjects in the Control group achieved a significant reduction of gingivitis only at the 3-month visit (5.7%, Table 4), but not at the 6-month visit (2.5%, Table 5). When the two groups were compared, no significant differences were detected at any of the visits, indicating the benefits of reducing gingivitis by the Colgate Sensitive Pro-Relief toothpaste, if any, was slight.

The present study also included a comprehensive examination of oral soft and hard tissues, which found no evidence of any abnormalities or adverse changes of the tissues throughout the 6-month period.

In addition, when questioned, none of the subjects reported any experience of adverse effects, related or non-related to the study products, at any of the clinical visits.

In conclusion, after 3 and 6 months of product use, there was no statistically significant difference between Colgate Sensitive Pro-Relief toothpaste and Colgate Cavity Protection fluoride toothpaste without arginine with respect to supragingival calculus formation. Additionally, after 3 and 6 months of product use, there was no statistically significant difference between Colgate Sensitive Pro-Relief toothpaste and Colgate Cavity Protection fluoride toothpaste without arginine with respect to gingivitis.

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