JOURNAL OF DENTISTRY 41S (2013) S22–S28

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The anti-caries efficacy of a dentifrice containing 1.5% arginine and 1450 ppm fluoride as sodium monofluorophosphate assessed using Quantitative Light-induced Fluorescence (QLF)

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ARTICLE INFO

Article history:
Received 26 February 2010
Received in revised form
6 April 2010
Accepted 7 April 2010

Keywords:
Arginine
Caries
Fluoride
Dentifrice
Arrest
Reversal
Enamel
Lesions

ABSTRACT

Objective: To compare the efficacy of a new dentifrice containing 1.5% arginine, an insoluble calcium compound and 1450 ppm fluoride to arrest and reverse naturally occurring buccal caries lesions in children relative to a positive control dentifrice containing 1450 ppm fluoride alone.

Study design: Participants from Chengdu, Sichuan Province, China tested three dentifrices: a new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride, as sodium monofluorophosphate, a positive control dentifrice containing 1450 ppm fluoride, as sodium fluoride, in a silica base, and a matched negative control dentifrice without arginine and fluoride. Quantitative Light-induced Fluorescence (QLF) was used to assess buccal caries lesions at baseline and after 3 and 6 months of product use.

Results: 438 participants (initial age 9–13 years (mean 11.1 ± 0.78) and 48.6% female) completed the study. No adverse events attributable to the products were reported during the course of the study. The subject mean ΔQ (mm^2 %), representing lesion volume, was 27.26 at baseline. After 6 months of product use, the ΔQ values for the arginine-containing, positive and negative control dentifrices were 13.46, 17.99 and 23.70 representing improvements from baseline of 50.6%, 34.0% and 13.1%. After 6 months product use, the differences between the pair wise comparisons for all three groups were statistically significant ($p < 0.01$). The arginine-containing dentifrice demonstrated an improvement after only 3 months that was almost identical to that achieved by the conventional 1450 ppm fluoride dentifrice after 6 months.

Conclusion: The new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride provides statistically significantly superior efficacy in arresting and reversing buccal caries lesions to a conventional dentifrice containing 1450 ppm fluoride alone.

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0300-5712
doi:10.1016/j.jdent.2010.04.004

1. Introduction

There is no doubt that the introduction of fluoride products, such as dentifrice and mouth rinses, has played a significant role in the dramatic decline in the prevalence and severity of dental caries in many geographies, particularly in developed countries.[1,2] Despite this success, dental caries remains a prevalent oral disease, and cavities remain a global public health problem.[1,3]

The past two decades of caries research has significantly advanced our knowledge of dental caries, and, in particular, has identified that caries is a dynamic, multi-factorial disease process involving the interface of the undisturbed plaque biofilm and the tooth surface when dietary sugars are present.[4–6] The process begins when acid-producing species, such as the mutans streptococci, within the undisturbed plaque biofilm metabolize these dietary sugars to produce lactic and other acids, causing initial de-mineralization, i.e., the first loss of calcium and phosphate ions from the hydoxyapatite structure of the tooth's enamel. This initial step results in a reversible early caries lesion which can be re-mineralized. Fluoride works by promoting re-mineralization of this de-mineralized tissue, reversing the caries process and adding calcium and phosphate ions back into the hydroxyapatite structure, as well as incorporating fluoride, as fluorapatite, to strengthen the mineral lattice.[7,8] If de-mineralization is left unchecked, however, dental caries can progress through various irreversible stages of enamel breakdown to frank cavitation.[9]

Importantly, this new knowledge has led to an understanding that caries is a disease continuum.[1] This, in turn, has begun to change clinical dentistry from a focus on restoration of cavities to investigation of therapeutic approaches to arrest or reverse the caries process by re-mineralizing initial enamel or root caries lesions that are not cavitated.[10] In large part, however, such interventions have focussed on mechanical plaque control measures to remove the biofilm and reduce acid production by cariogenic bacteria, and treatment with fluoride to facilitate re-mineralization and inhibit de-mineralization.[11] Dentifrice is an ideal vehicle for fluoride because it simultaneously facilitates plaque removal during brushing and ensures effective delivery of fluoride for the treatment and prevention of carious lesions. In fact, fluoride dentifrice is the most effective, evidence-based caries preventive measure available today.[12]

During the past decade, a new approach to the management of dental caries has been identified and validated. This new approach combines arginine and an insoluble calcium compound with fluoride to complement and enhance the effects of fluoride by targeting the first step in the caries process, i.e., the first acid attack, modulating the metabolism of the plaque biofilm, neutralizing plaque acids and, thereby, reducing the harmful effects of the plaque biofilm.[1,13] The arginine is metabolized to ammonia through the arginine deiminase pathway in non-pathogenic arginolytic organisms, such as *Streptococcus sanguis*. In turn, this ammonia neutralizes plaque acids and stabilizes the residual plaque biofilm, preventing a shift in the oral flora to aciduric bacterial species and maintaining a non-cariogenic plaque after sugar challenge.[1,13,14] Thus, the less acidic intra-oral pH encourages re-mineralization and reduces de-mineralization.[15] In addition, the insoluble calcium compound is able to provide free calcium ions to supplement the re-mineralization process.[1,13,14,16']

The anti-caries benefits of a new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride, as sodium monofluorophosphate (MFP), have been demonstrated in a series of clinical studies involving reversible caries lesions in adults, as well as in children.[17–20]

In two studies, the ability of the new arginine-containing dentifrice to re-mineralize non-cavitated primary root caries lesions was evaluated. In one study, the new dentifrice was compared to that of a positive control dentifrice containing 1450 ppm fluoride, as sodium fluoride, in a silica base, and a fluoride-free, matched negative control. After 6 months use of the new dentifrice, one lesion (0.7%) became worse and 61.7% of subjects had lesions which hardened. In contrast, for the positive and negative control dentifrices, 9.0% and 18.2% of subjects became worse, and 56.0% and 27% improved, respectively. The differences between the new arginine-containing dentifrice and both the positive and negative control dentifrices were statistically significant ($p < 0.01$).[17] In the other study, the new dentifrice was compared to that of a matched positive control dentifrice containing 1450 ppm fluoride alone. After 6 months use of the new dentifrice, 70.5% of subjects had lesions which hardened compared to 58.1% of subjects in the positive control group. The difference in the number of lesions being hardened between the new arginine-containing dentifrice and the positive control dentifrices was statistically significant ($p < 0.05$).[18]

In three studies, including the study reported in this paper, the benefits of the new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride in arresting and reversing buccal enamel carious lesions were demonstrated using Quantitative Light-induced Fluorescence (QLF) methods to measure changes in mineralization of enamel lesions.[19,20] The Quantitative Light-induced Fluorescence (QLF) method is based upon the principle that a tooth fluoresces green when it is stimulated with blue light. Emitted green light is scattered within an early lesion, when caries is present, such that the overlying tooth surface appears dark against a green background. By comparing the loss of fluorescence, due to scattering in the lesion, to the background level of fluorescence, both the area and degree of de-mineralization (ΔF) of the lesion can be quantified. In addition, the volume of the lesion (ΔQ) can be estimated by multiplying the lesion area by the loss of fluorescence (ΔF). This method has now been employed in a number of clinical studies.[21–24]

In one study, the new dentifrice was compared to two matched dentifrices, one with neither fluoride nor arginine as a negative control and the other a 1450 ppm fluoride only dentifrice as a positive control. After 6 months use of the products, the mean lesion size was reduced by 50.7% for the arginine-containing dentifrice, 32.3% for the positive control and 11.4% for the negative control. The differences between the arginine-containing dentifrice and both the positive and negative control dentifrices were statistically significant ($p < 0.01$).[19] In the other study, the new dentifrice was compared to a matched 1450 ppm fluoride dentifrice as a

positive control. After 6 months product use, the mean lesion size was reduced by 44.6% for the arginine-containing dentifrice and 28.9% for the positive control. Again, the differences between both the arginine-containing dentifrice and the positive control dentifrice were statistically significant ($p < 0.05$).[20]

Finally, a 2-year conventional caries clinical study has proven that two dentifrices containing 1.5% arginine and 1450 ppm fluoride in a calcium base, one with di-calcium phosphate and the other with calcium carbonate, are significantly more effective in preventing the formation of cavitated caries lesions than a dentifrice containing 1450 ppm fluoride alone. Three trained and calibrated dentists examined the children at baseline and after one and two years using the National Institute of Dental Research Diagnostic Procedures and Criteria. The number of decayed, missing, and filled teeth (DMFT) and surfaces (DMFS) for the three study groups were very similar at baseline, with no statistically significant differences among groups. After one year, there were no statistically significant differences in caries increments among the three groups. After two years, the two groups using the dentifrices containing 1.5% arginine, an insoluble calcium compound and 1450 ppm fluoride had statistically significantly ($p < 0.02$) lower DMFT increments (21.0% and 17.7% reductions, respectively) and DMFS increments (16.5% and 16.5%) compared to the control dentifrice. The differences between the two groups using the new dentifrices were not statistically significant. The results of this pivotal clinical study support the conclusion that dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride provide superior protection against caries lesion cavitation to dentifrices containing 1450 ppm fluoride alone.[25]

The aim of this 6 month study was to confirm the hypothesis that the new dentifrice containing 1.5% arginine and 1450 ppm fluoride is more effective in arresting and reversing naturally occurring buccal caries lesions in children than a regular dentifrice containing fluoride alone. Three dentifrices were investigated: an arginine-containing dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride, as sodium monofluorophosphate, a positive control dentifrice containing 1450 ppm fluoride, as sodium fluoride, in a silica base and a matched negative control dentifrice with neither fluoride nor arginine.

2. Methods

This study was a randomised, controlled, double blind clinical trial of 6 month's duration which compared a new arginine-containing dentifrice to both positive and negative controls. The three products investigated in this study were

1. Arginine-containing dentifrice: 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride as sodium monofluorophosphate (Colgate-Palmolive Company, New York, NY).
2. Positive control: 1450 ppm fluoride as sodium fluoride in a silica base (Colgate-Palmolive Company, New York, NY).
3. Negative control: non-fluoride dentifrice with a matched calcium base (Colgate-Palmolive Company, New York, NY).

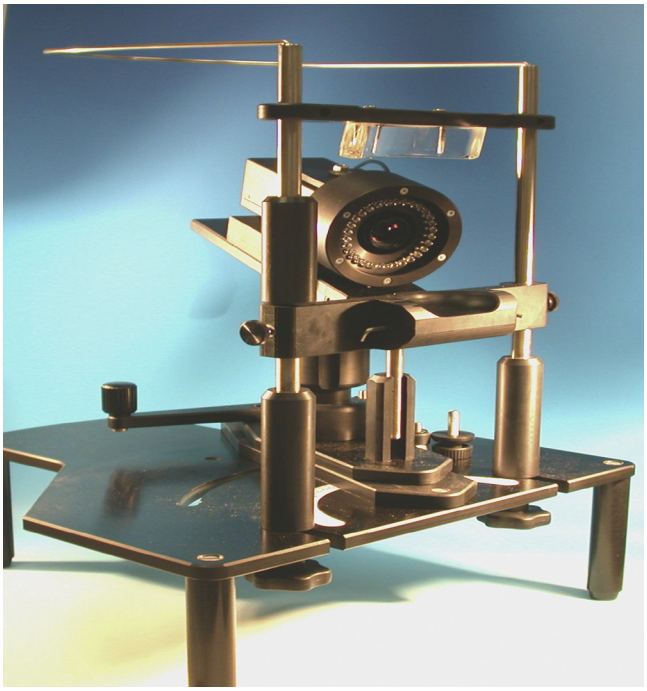

Fig. 1 – Set up of the QLF system.

The study received ethical approval from the Institutional Review Board of Sichuan Province Committee for Oral Health.

The study participants were from five primary schools in Chengdu, Sichuan Province, China, which has a water supply containing 0.3 ppm fluoride. The schools were selected to have similar socio-demographic profiles and also on their ability to provide supervised brushing to the participants. Participating subjects were aged 9–13 years at the start of the study and had to have at least one visible white spot lesion on the buccal surface of one of the upper six anterior teeth. They were also required to have a signed consent form from a parent or guardian. Subjects meeting the screening criteria were randomly allocated to groups by the study administrator.

After the baseline and 3 months examinations, two individually labelled white laminated tubes of the assigned dentifrice and a Colgate Extra Clean toothbrush (Colgate-Palmolive, New York, NY, USA) were given to each study participant. Only one type of dentifrice was assigned per family and additional tubes of dentifrice were available on request from participants. Participants were given oral hygiene instruction and advised to brush at least twice per day (morning and evening) with the toothbrush and dentifrice supplied. During school days, participants brushed in the afternoon with their allocated dentifrice for 2 min under the supervision of the school nurse.

Assessments were performed at baseline, and after 3 and 6 months of product use, in the school nurse's office. This room was darkened to control ambient lighting. For each subject between three and five views were taken of the upper anterior teeth so that good quality images could be captured of each lesion. Prior to image capture, the teeth were dried with compressed air for five seconds. Images were captured using a custom built, high resolution, fluorescence imaging system. This has been described previously in some detail.[26]

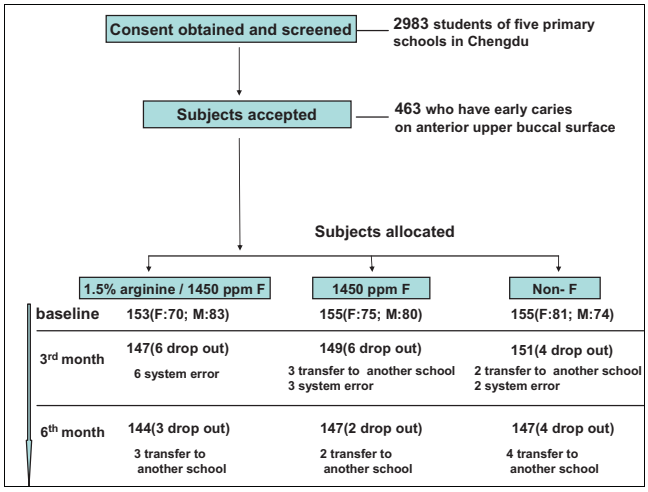

Fig. 2 – Disposition of subjects.

The camera and illuminator were mounted in a geometry stabilising unit (Fig. 1). This, together with video repositioning software, enabled subjects to be accurately repositioned at each visit. Images were acquired using QLF Patient software (Inspektor Research Systems BV, Amsterdam, The Netherlands). For the 3 and 6 months visits, the video repositioning software was used with a grab level of 0.95. Images were analysed to calculate lesion area and loss of fluorescence using QLF 2.00 software (Inspektor Research Systems BV). All images were taken by the same examiner.

2.1. *Statistical analysis*

The QLF software was used to calculate the area (mm^2) and percentage loss of fluorescence (ΔF%) of the lesion using a threshold for difference of 5% from the reconstructed image. The ΔF and area were used to calculate ΔQ (mm^2 %) which indicates the volume of the lesion.

The primary outcome for this study was the mean subject ΔQ at the 6-month examination. The three study groups were compared using a Linear Model controlling for baseline ΔQ and number of lesions per subject. Other statistical comparisons were based on a similar model with the baseline QLF metric and number of lesions per subject as covariables. A Bonferroni adjustment was applied to all pair wise comparisons.

3. Results

3.1. *Reproducibility*

At the baseline examinations, 27 randomly selected children were examined using the QLF system and repeat examinations were conducted 24 h later without using the video repositioning (Vidrep) function. The Intra-class Correlation Coefficient (ICC) between the two examinations was 0.95. In addition, to check the reproducibility of the software analysis, 32 subjects were randomly selected and a repeat analysis conducted after a 1-week interval. An ICC value of 0.93 was reached for the ΔQ values.

3.2. *Disposition of subjects*

A total of 2983 children were consented to take part in the study and were screened. Of these 463 satisfied the inclusion and exclusion criteria and were recruited into the study. At the 3-month examination, sixteen subjects were lost from study. Of these, five transferred to another school; for eleven subjects, images could not be captured at the time of each scheduled assessment. At the 6-month examination, a further nine subjects, who transferred to another school, were lost from the study (Fig. 2).

A total of 438 children completed the study. Of these, 144 were in the arginine-containing dentifrice, 147 in the positive control and 147 in the negative control groups. The children were aged 9–13 years (mean 11.1 ± 0.78) at the start of the study and 48.6% were female. No adverse events were reported in any of the study groups during the course of the study.

3.3. *Clinical results*

For each subject, a mean of 3.35 (±1.18) lesions were imaged (Table 1). The maximum number of lesions per subject imaged was six and minimum one. At the baseline examination, the three study groups were well balanced with respect to all QLF metrics with no statistically significant differences between the groups (Table 1).

3.4. *Three months*

For ΔF, the baseline mean was 9.16% and after 3 months was 8.23, 8.50 and 8.67 for the arginine-containing, positive and

Table 1 – Subject mean (SD) QLF metrics (ΔF, Area and ΔQ) at baseline and 3 and 6 months for the three study groups and number of lesions at baseline.

Group	N lesions/subject	Baseline			3 months			6 months		
		ΔF	Area	ΔQ	ΔF	Area	ΔQ	ΔF	Area	ΔQ
1.5% arginine/1450 MFP	3.44 (1.19)	9.17 (1.96)	2.48 (1.79)	26.75 (25.91)	8.24 (1.83)	1.79 (1.44)	17.64 (18.12)	7.95 (1.82)	1.40 (1.14)	13.12 (13.08)
1450 NaF	3.29 (1.19)	9.24 (2.16)	2.60 (1.98)	28.00 (26.83)	8.56 (2.12)	2.05 (1.77)	21.24 (22.75)	8.43 (2.07)	1.83 (1.50)	18.46 (18.43)
Non-F	3.33 (1.17)	9.06 (1.82)	2.50 (1.86)	27.02 (29.23)	8.60 (1.83)	2.29 (2.14)	24.32 (29.23)	8.48 (2.24)	2.16 (2.24)	23.54 (29.84)

Table 2 – Baseline adjusted subject mean (SE) QLF metrics (ΔF, Area and ΔQ) at 3 and 6 months for the three study groups with number of lesions at baseline as covariable.

Group	Adjusted 3 months			Adjusted 6 months		
	ΔF	Area	ΔQ	ΔF	Area	ΔQ
1.5% arginine/1450 MFP	8.23 (0.11)	1.83 (0.08)	18.00 (1.07)	7.94 (0.12)	1.44 (0.09)	13.46 (1.07)
1450 NaF	8.50 (0.11)	1.99 (0.08)	20.71 (1.06)	8.37 (0.12)	1.78 (0.09)	17.99 (1.06)
Non-F	8.67 (0.11)	2.31 (0.08)	24.50 (1.06)	8.56 (0.12)	2.18 (0.09)	23.70 (1.05)
Baseline mean	9.16	2.53	27.26	9.16	2.53	27.26
N lesions			3.35			

negative control dentifrices, respectively (Table 2). For lesion area, the baseline mean was 2.53 mm^2 compared to 1.83, 1.99 and 2.31 for the arginine-containing, positive and negative control dentifrices, respectively, at the 3 months examination. For ΔQ, the baseline mean was 27.26 mm^2 % and after 3 months was reduced to 18.00, 20.71 and 24.50 representing improvements from baseline of 34.0%, 24.0% and 10.1% for the arginine-containing, positive and negative control dentifrices, respectively. The differences between the negative control dentifrice and the both the arginine-containing and positive control dentifrices were statistically significant.

3.5. *Six months*

For the arginine-containing, positive and negative control dentifrices, the baseline adjusted mean ΔF values at the 6-month examination were 7.94, 8.37 and 8.56 and for area were 1.44, 1.78 and 2.18. Compared to the baseline mean value for ΔQ of 27.26 mm^2 %, the values at 6 months had reduced for ΔQ to 13.46, 17.99 and 23.70 (Table 2). This represents improvements from baseline of 50.6%, 34.0% and 13.1% for the arginine-containing dentifrice, and the positive and negative control dentifrices, respectively. The baseline adjusted ΔQ data are shown in Fig. 3. It can be seen that the improvement in the arginine-containing dentifrice group at 3 months is comparable to that at 6 months for the fluoride-containing positive control dentifrice. For ΔQ, the differences between the negative control group and both the arginine-containing dentifrice and the positive control groups were statistically significant ($p < 0.001$). Further, the difference between the arginine-containing dentifrice and positive control groups was also statistically significant ($p = 0.003$). Fig. 4 plots the total percentage of subjects with improvements from baseline as a function of the percentage mean improvement for the three groups. It can be seen that 88.9% of subjects showed some improvement (reduction in mean ΔQ) in the arginine-containing dentifrice group compared to 78.9% in the positive control and 70.7% in the negative control groups. In nearly half (47.2%) of the subjects using the arginine-containing dentifrice, the lesions reduced in size by 50% or more compared to only 39.5% of the subjects in the positive control group and 34% in the negative control group.

4. Discussion

This study has provided further clinical evidence that a new dentifrice containing 1.5% arginine, an insoluble calcium

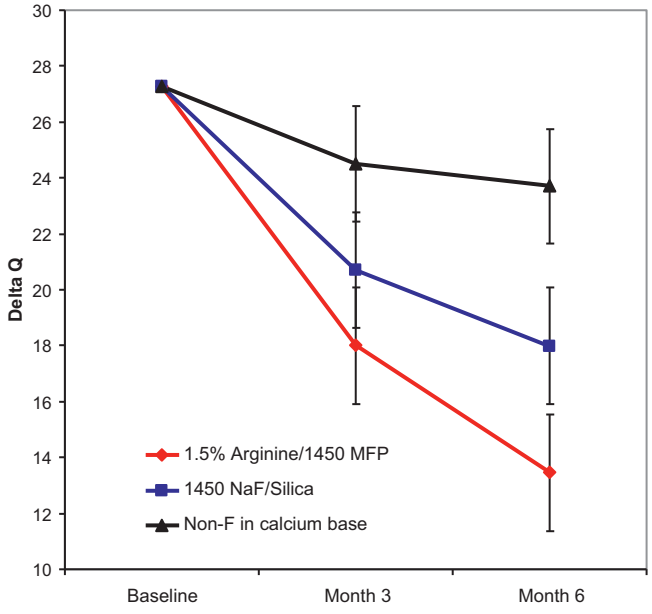

Fig. 3 – Mean ΔQ (representing lesion volume in mm^2 %) outcomes at baseline, 3 and 6 months with 95% confidence intervals.

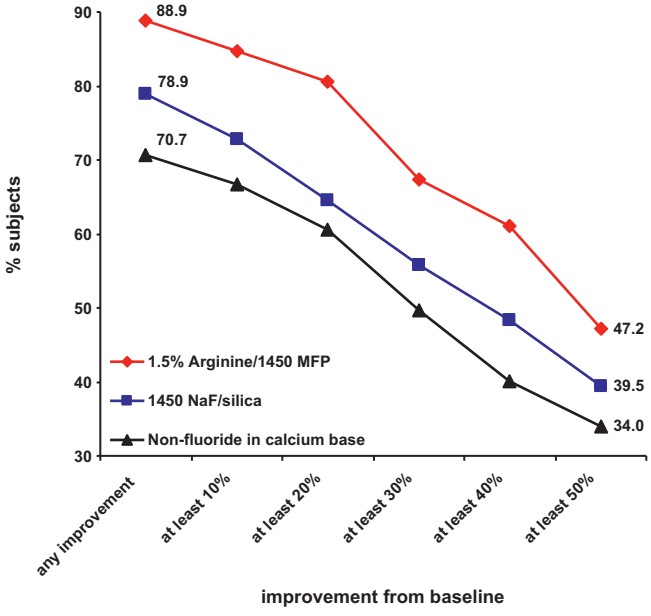

Fig. 4 – Total frequency of subjects showing a reduction in mean lesion size bay at least 0–50% after 6 months product use for the three study groups.

compound, and 1450 ppm fluoride, as MFP, provides anti-caries efficacy that is significantly superior to that of conventional 1450 ppm fluoride dentifrices. After 6 months use of the arginine-containing product, lesions had reduced in mean size from baseline by 50.6% compared to only 34.0% reduction after 6 months use of the conventional 1450 pm fluoride dentifrice. 34.0% reduction in lesion size was achieved after just 3 months use of the arginine-containing dentifrice indicating that lesions were re-mineralizing twice as quickly with the arginine-containing dentifrice as with the conventional fluoride dentifrice. Thus, this study confirmed the hypothesis that the new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride is more effective than a positive control dentifrice containing 1450 ppm fluoride alone in arresting and reversing naturally occurring buccal caries lesions in children.

In the two other studies conducted on this new dentifrice, using the QLF method, very similar results were observed, with reductions in lesion area of approximately 50% and 45% in the two groups using the dentifrice containing 1.5% arginine, and insoluble calcium compound, and 1450 ppm fluoride, and approximately 32% and 29% in the two groups using the 1450 ppm fluoride positive control dentifrices.[19,20] In the one of the studies, a fluoride-free matched negative control dentifrice was also included to assess the effect of brushing with dentifrice without fluoride. An approximately 11% reduction in lesion size was attributable to improved plaque control at the 6 months examination in that study.[19]

The results of these studies show that the new arginine-containing dentifrice provides an enhancement in efficacy in reversing early enamel lesions over dentifrices with fluoride alone that is numerically comparable to the enhancement that a 1450 ppm fluoride dentifrice provides over a fluoride-free dentifrice. As the clinical efficacy of fluoride toothpaste in preventing cavity formation is well established in traditional caries clinical trials (fluoride compared to non-fluoride toothpaste),[12] the clinical implication of these results is that, with continued use, the arginine-containing dentifrice will provide substantially more effective cavity prevention than dentifrices containing fluoride alone, by virtue of its enhanced ability to arrest and reverse early lesions, thereby preventing their progression to irreversible lesions and cavities.

Based upon the results of the three studies conducted on the new arginine-containing dentifrice, as well as of several studies validating the QLF method by distinguishing products that had previously been investigated in conventional 2–4 years caries clinical studies, it is apparent that the use of QLF in studies of early coronal caries significantly improves the ability to discriminate between anti-caries products enabling the studies to be conducted effectively in shorter time periods with fewer participants. It further appears that the ability of products designed to enhance the re-mineralization of initial carious lesions is measured accurately, prior to stages of cavitation, enabling quantification of their efficacy under conditions most appropriate to their mechanisms of action. It is known that therapeutic treatment of initial carious lesions is both possible and practical, and by employing sophisticated detection and measurement methods, such as QLF, this approach is facilitated and outcomes can be monitored so that participants receive optimum, minimally invasive treatment. Thus, the QLF method has proven to be a powerful tool in the caries clinical hierarchy providing unique insights regarding efficacy and mechanism of action of anti-caries products on early coronal caries lesions that complement and augment those generated in conventional 2-year caries studies of cavitation.

5. Conclusion

It is concluded that both fluoride-containing dentifrices are significantly better at arresting and reversing buccal caries lesions than the non-fluoride dentifrice. More importantly, the new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride provides significantly greater benefit in arresting and reversing buccal caries lesions than the conventional fluoride dentifrice.

Conflict of interest statement

Drs Zhang, Cummins and Ellwood are employees of the Colgate-Palmolive Company. Drs Yin, Hu, Li, Fan and Pretty have no conflict of interest. Mr Mateo provided independent statistical review of the data for the Colgate-Palmolive Company on a consultancy basis.

Acknowledgement

The author, Y.W. acknowledges support from the China Scholarship Council (2007102764).

This study was sponsored by the Colgate-Palmolive Company.

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