

A study into the plaque-inhibitory activity of experimental toothpaste formulations containing antimicrobial agents

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Abstract

Background/Aims: The use of specific antimicrobial agents in toothpastes may help reduce plaque and gingivitis. There would also appear to be some value in formulating products that contain combinations of such agents that may potentiate any activity present. The aims of this exploratory and pragmatic study were twofold:- (1) exploratory: to compare the effects on plaque re-growth of two zinc citrate/triclosan formulations, one of which contained bromochlorophene and hence demonstrate any additional beneficial effects produced by the addition of the phenol. (2) pragmatic: to assess whether both pastes were significantly better than a benchmark control, proprietary fluoride toothpaste at inhibiting plaque formation.

Methods: Following an initial prophylaxis to remove all plaque and calculus, toothpaste slurry rinses were used over a 96 h period by 24 volunteers, while omitting all other oral hygiene procedures. After 24, 48 and 96 h, plaque was measured by plaque area and by plaque index. For comparative purposes, a conventional commercial fluoride toothpaste rinse was also used as a benchmark control in this triple cross-over double-blind study.

Results: With one exception, comparisons between the three pastes failed to show any significant differences in plaque accumulation at 96 h whether assessed by plaque index or area. At this time period, significantly more plaque was seen with the zinc citrate paste without bromochlorophene, compared with that of the control paste.

Conclusions: The findings from this study failed to demonstrate a plaque-inhibitory action from the two novel formulations beyond that of a conventional benchmark toothpaste, although overall levels of plaque formed by the volunteers, especially on the control paste were generally lower than in previous studies. Nevertheless, it remains to be determined whether the test formulations could exert a direct anti-inflammatory action against gingivitis by way of the triclosan delivery system. Neither test formulation was subsequently marketed.

Key words: bromochlorophene triclosan; clinical trial toothpaste; plaque inhibition; zinc citrate

The use of therapeutic agents in toothpaste and mouthrinses to reduce or inhibit plaque formation is now a well-established approach to improving gingival health (Kornman 1986, van der Ouderaa 1991, Addy & Renton-Harper 1996). One of the earliest groups of compounds studied was metal salts

(Hanke 1940). Of these salts, zinc and tin have received the greatest attention, more so because of their recognized antibacterial activity and their relative high safety profiles (Scheie 1989, for a review, see Jackson 1997). More recently, another 'active' ingredient triclosan has been used in mouth rinses

and toothpaste. This compound is usually used in combination with zinc citrate or the copolymer PVM/MA, the former to potentiate the antibacterial activity of the formulation and the latter to improve oral retention of the triclosan. With these products, some success has been claimed for inhibition of plaque and

reduction of gingivitis and calculus (Gjerme & Saxton 1991, Svaton et al. 1993, for reviews see Cummins 1997, Jackson 1997). In addition to possible plaque-inhibitory activity, there is evidence that triclosan possesses an anti-inflammatory action (Gaffar et al. 1995) and this may produce additional benefits to gingival health that are not necessarily related to control of plaque (Waele et al. 1993, Kjaerheim et al. 1996, Skaare et al. 1996). At present, there is little information available on the potential value of using other less well-known antimicrobials in toothpaste products. Bromochlorophene is one such antimicrobial, which is known to be an effective bactericide and a potential plaque-inhibitory agent. Clinically a fluoride/bromochlorophene toothpaste was shown to reduce caries in both animals and humans (Kinkel & Stolte 1968) and also to induce significant reductions in plaque growth for both area and index, when measured 24 h after a single use (unpublished data). There is, however, sparse published clinical evidence to show that toothpastes containing bromochlorophene have an inhibitory effect on plaque accumulation. One clinical study of a triglyceride oil/zinc citrate/bromochlorophene toothpaste did demonstrate a 24% reduction in plaque compared with a conventional fluoride paste using the same methodology as presented here (Moran et al. 2001).

The design of this randomized, controlled, cross-over, clinical trial was planned to be both exploratory and pragmatic. Firstly, two zinc citrate/triclosan formulations with and without bromochlorophene were compared in a 4-day plaque re-growth model to explore the potential additional value of bromochlorophene for plaque inhibition. Secondly, both test formulations were assessed for plaque inhibition in a pragmatic design by comparing with a benchmark control, proprietary fluoride toothpaste.

Materials and Methods

Plaque-inhibitory efficacy alone was measured in this study using a single-centre, double-blind, three-treatment, randomized cross-over design. The subjects were randomly allocated to each of six possible treatment orders. Using a six 3×3 Latin square design, each treatment appeared equally frequently in each period, resulting in balance for

carryover, which would negate any influence of a washout period of 3 days being insufficient. The test toothpastes contained both zinc citrate at 0.5% and triclosan at 0.14% adsorbed onto polymer spheres. One of the pastes also contained bromochlorophene at 0.1%, added to enhance any plaque-inhibitory activity of the paste. These non-commercial pastes were matched as far as possible with the control paste with regard to taste and colour and were dispensed out of sight of each volunteer. The benchmark control (Addy et al. 1992) paste was a commercial fluoride product (Colgate Ultra Cavity Protection, Colgate-Palmolive Limited, Guildford, Surrey, UK), which contained none of the aforementioned ingredients. The study was conducted in accordance with the Declaration of Helsinki (1964) and subsequent amendments. Prior to the start of the study, ethical approval was obtained from the United Bristol Health Care Trust and volunteers provided signed and witnessed consent to participate. The study was designed, conducted, analysed and reported according to guidelines for Good Clinical Practice.

A total of 24 volunteers took part (11 males, 13 females, age range 20–35 years). The volunteers were dentate with a catalogued high standard of oral hygiene and gingival health. Volunteers who wore fixed or removable appliances or dental prostheses were excluded, as were those with any medical or pharmacological history that could compromise the conduct of the study. The subjects were given a conventional fluoride dentifrice (Colgate-Palmolive Limited) and toothbrush (Boots Adult; Boots, Nottingham, UK) to use in place of their normal products 1 week before the study and during the wash-out phase. A week-end wash-out phase from Friday evening to the following Monday was allowed between each treatment period. On day 1 (Monday) of each trial period, the subjects had their plaque disclosed with D&C Red #28 dye and were then given a dental prophylaxis to clean their teeth free of plaque and calculus deposits. After this, they were provided with toothpaste and toothbrush dispensed by an assistant who had previously applied a 1 cm ribbon of toothpaste to the brush out of sight of each volunteer. They were then told to thoroughly brush their teeth for 30 s. Following this they were instructed to return to the clinic that afternoon and under supervision were

told to rinse their mouths for 1 min. with 10 ml of the allocated toothpaste slurry (3 g/10 ml), again previously prepared out of sight of the volunteer. For the following 3 days at the same times, in the morning and afternoon, they repeated the rinsing procedure. During this period the subjects were instructed not to use any other forms of oral hygiene, i.e. toothbrushing, flossing, etc. Twenty-four hours after the initial prophylaxis (Tuesday) the volunteers returned to the clinic when their teeth were disclosed with the aforementioned dye. The area of plaque on the buccal surfaces of the upper and lower incisors, canines and pre-molars was drawn onto standard tooth charts according to the method described by Addy et al. (1983). The area of plaque on each tooth was then measured with a digitizer in conjunction with a laptop microcomputer and DCAD software package. In addition, plaque on the buccal and lingual surfaces of the teeth was assessed using the Turesky et al. (1970) modification of the Quigley & Hein (1962) plaque index. Subsequently both methods of plaque assessment were used again at 48 h (Wednesday) and 96 h (Friday) after the initial prophylaxis. During the following weekend the subjects used the toothbrush and wash-out dentifrice provided at home, in place of their normal toothbrush and dentifrice.

Statistical analyses

The primary analysis compared the whole-mouth mean plaque score and plaque area values. Evaluation of eight subsets of sites, e.g. all upper surfaces, all lower surfaces, all anteriors, all posteriors, etc. was also carried out but are not reported here as they showed essentially the same picture. Because of an incomplete data set simple means for each treatment required a slight adjustment for confounding with variation between subjects or between treatments. The distribution for plaque index did not display consistent evidence of skewness and analyses based on the Gaussian distribution without transformation were deemed appropriate. For both plaque index and area the analysis of variance (ANOVA) model was used to determine the effects of treatment, subject and period. Contrasts between treatments were assessed by determining point estimates and 95% confidence intervals. Because of considerable positive skewness of measurements, confir-

matory non-parametric (Wilcoxon's) analyses were used for plaque area.

Results

Of the 24 subjects who started the study, 22 finished with a complete set of data. No untoward side effects were noted. The mean whole-mouth plaque scores and plaque area on the buccal surfaces of the teeth at 24, 48 and 96 h are shown in Tables 1 and 2. The use of the smaller screen size on this computer resulted in lower plaque area measurements (reduced by a factor of $\times 10.638$) compared with those reported in previous studies by this group.

Statistical comparisons for each time period between test and control toothpastes are shown in Tables 3 and 4. ANOVA showed a highly significant variation between subjects ($p < 0.001$) at 24, 48 and 96 h for both plaque score and area. There was also some evidence for differences in plaque between periods, especially at 96 h, with levels decreasing as the study progressed. Essentially, the amounts of plaque recorded at each time interval were similar for all the three toothpastes. Neither of the test pastes was statistically more effective than the control paste at inhibiting plaque, although numerically the zinc/bromochlorophene paste was the most effective of the three pastes at 96 h when assessed using the Turesky et al. (1970) index. However, differences between this paste and the other two were not statistically signifi-

cant. The test paste containing only the zinc citrate was consistently the least effective paste of the three at all time intervals. This lack of activity is reflected in the statistical analyses. These showed that there was significantly less plaque with the control paste compared with the zinc citrate paste at 24 h ($p < 0.05$) and 96 h ($p < 0.007-0.016$) when measured by the plaque area and at 24 h measured by the plaque index ($p < 0.05$). All other comparisons between the three pastes failed to show significant differences between the three pastes at all time intervals, whether assessed by plaque area or plaque index.

Discussion

This study was designed to compare the effects on plaque re-growth of two experimental toothpastes both containing 0.5% zinc citrate. Additionally one of the test pastes contained bromochlorophene which could confer additional antimicrobial activity resulting in further effects on plaque. It was also deemed appropriate to determine whether both pastes exhibited any plaque-inhibitory activity beyond that of a conventional 'benchmark' fluoride control paste (for a review see Addy et al. 1992). Both of the test pastes also contained triclosan at 0.14%, incorporated within porous polymer spheres.

Although the triclosan used was at a lower concentration than that usually seen in current commercial pastes, these spheres may enhance the delivery of the

triclosan to the oral soft tissues for more effective targeting of the reported anti-inflammatory activity of this agent (Gaffar et al. 1995, Mustafa et al. 1998). Thus, the use of the triclosan in this delivery system in the test pastes was not intended to primarily produce an additional plaque-inhibitory effect, but was added to exert an anti-inflammatory effect independent of any perceived effect on plaque. Clearly the effect of the triclosan as an anti-inflammatory agent could not be tested in the present model. Also, the design could not evaluate any plaque-inhibitory effect of this low level of triclosan. Indeed from the outset, this was not one of the aims of the study. These additives were used in the formulations together with usual toothpaste ingredients such as humectants, flavouring and the detergent sodium lauryl sulphate, the latter also known to have antibacterial and plaque-inhibitory activities (Addy et al. 1983, Moran & Addy 1984, Jenkins et al. 1991). Thus the pragmatic design of the study was not to demonstrate the efficacy of any potentially active ingredient in isolation but to show that the product as a whole was significantly better than a recognized benchmark fluoride toothpaste, for which no plaque-inhibitory action had been claimed. The findings of this study would suggest that neither of the experimental pastes conferred additional plaque-inhibitory effects beyond that of the conventional fluoride paste. In terms of concentration, the levels of the zinc salt should have been optimal for an antimicrobial effect. Both the test pastes contained zinc citrate at 0.5%, which was comparable with zinc citrate toothpastes assessed in previous studies (Saxton et al. 1986, Jones et al. 1988, for a review see Jackson 1997). It is interesting to note that a previous study of a zinc citrate toothpaste, again employed as a toothpaste slurry rinse failed to show reduced plaque formation beyond a conventional fluoride toothpaste (Addy et al. 1989). Previous evidence has also suggested that zinc has only a small effect on plaque growth on teeth that had originally been cleaned (Addy et al. 1980, Saxton et al. 1988). It is apparent that the major effect of zinc would appear to be on existing plaque in which the rate of bacterial proliferation in the plaque is reduced.

The exploratory arm of the study used bromochlorophene, incorporated into one of the test formulations, to

Table 1. Whole-mouth plaque score (Turesky et al. 1970) comparing two test toothpastes with a conventional control toothpaste

Time (h)	Mean + s.d.		
	Toothpaste A	Toothpaste B	Toothpaste C
24	1.09 (0.40)	1.06 (0.45)	1.14 (0.36)
48	1.55 (0.44)	1.52 (0.56)	1.62 (0.44)
96	2.10 (0.52)	2.16 (0.55)	2.25 (0.46)

A, zinc citrate/bromochlorophene/triclosan toothpaste; B, control fluoride toothpaste; C, zinc citrate/triclosan toothpaste.

Table 2. Mean plaque area ($\times 10^{-3}$) comparing two test toothpastes with a conventional control toothpaste

Time (h)	Mean + s.d.		
	Toothpaste A	Toothpaste B	Toothpaste C
24	8.0 (8.9)	7.5 (9.5)	10 (11.6)
48	17.1 (14.7)	15.1 (13.2)	20.3 (16.7)
96	45.6 (33.3)	41.6 (22.7)	53.9 (24.3)

A, zinc citrate/bromochlorophene/triclosan toothpaste; B, control fluoride toothpaste; C, zinc citrate/triclosan toothpaste.

Table 3. Whole-mouth Turesky index

	Point estimate	95% confidence interval	p-value
24 h			
A versus B	0.047	-0.043 to 0.137	0.30
C versus B	0.093	0.003 to 0.183	0.043
A versus C	-0.046	-0.135 to 0.043	0.30
48 h			
A versus B	0.043	-0.138 to 0.224	0.64
C versus B	0.107	-0.074 to 0.288	0.24
A versus C	-0.064	-0.239 to 0.111	0.46
96 h			
A versus B	-0.030	-0.193 to 0.134	0.72
C versus B	0.122	-0.041 to 0.285	0.14
A versus C	-0.151	-0.309 to 0.006	0.059

Contrasts between Toothpastes, adjusted for missing data.

A, zinc citrate/bromochlorophene/triclosan toothpaste; B, control fluoride toothpaste; C, zinc citrate/triclosan toothpaste.

Table 4. Plaque area

	Point estimate	95% confidence interval	ANOVA (p)	Wilcoxon (p)
24 h				
A versus B	0.0007	-0.0016 to 0.0029	0.55	0.26
C versus B	0.0027	0.0005 to 0.0049	0.019	0.015
A versus C	-0.0020	-0.0042 to 0.0002	0.069	0.22
48 h				
A versus B	0.0024	-0.0032 to 0.0080	0.40	0.49
C versus B	0.0056	-0.0001 to 0.0112	0.052	0.058
A versus C	-0.0032	-0.0086 to 0.0022	0.24	0.38
96 h				
A versus B	0.0055	-0.0042 to 0.0152	0.26	0.52
C versus B	0.0137	0.0040 to 0.0234	0.007	0.016
A versus C	-0.0083	-0.0176 to 0.0011	0.083	0.074

Contrasts between Toothpastes, adjusted for missing data.

A, zinc citrate/bromochlorophene/triclosan toothpaste; B, control fluoride toothpaste; C, zinc citrate/triclosan toothpaste.

determine whether this phenol would enhance plaque-inhibitory activity. Toothpastes incorporating this bactericide have been shown in vitro to be effective against plaque bacteria associated with periodontal disease and dental caries (Hardie 1991). Clinically, a fluoride/bromochlorophene toothpaste was shown to reduce caries in both animals and humans (Kinkel & Stolte 1968) and also to induce significant reductions in plaque growth for both plaque area and index, when measured 24 h after a single use (unpublished data). Recently, a triglyceride oil/zinc citrate/bromochlorophene toothpaste was shown to reduce plaque by 24% compared with the same control paste used in the present study (Moran et al. 2001). Relevant to these findings, such a paste has been marketed. The failure in the present study to show an effect over the minus bromochlorophene test paste is perhaps surprising and is at variance with the findings and conclusions drawn

from the previously mentioned study on a zinc citrate/bromochlorophene toothpaste (Moran et al. 2001). However, the test formulation in the previous study was not identical to the present formulation even though the concentrations of both the zinc citrate and bromochlorophene were the same.

The findings of the present study do not necessarily mean that the test pastes would not have any benefits on plaque or gingivitis. Even conventional non-'gum health' formulations, such as those used in the present study, may possess antibacterial properties because of ingredients such as detergents, which may reduce plaque formation compared with water controls (Addy et al. 1983, Moran & Addy 1984). Equally relevant, it is not possible from the design of the present study to determine the level of activity of the control paste. It is acknowledged, although, that the control paste plaque levels were numerically lower (by approximately 20% at

96 h for the Turesky index, and substantially lower for area measures by planimetry) to that of the same paste used in a previous study by the authors (Moran et al. 2001). In addition, some of the volunteers showed lower than expected levels of plaque than seen in previous trials, and therefore the use of different subjects with differing tendencies for plaque re-growth does not make a direct comparison possible. It therefore remains to be determined whether the new formulations would be of value to gingival health irrespective of a failure, in this study, to exhibit any effects on plaque formation. It should also be pointed out that these experimental formulations were not subsequently marketed.

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