INVESTIGATION OF TRICLOSAN RETENTION AND DENTAL PLAQUE VIABILITY WITH A TRICLOSAN/PVM/MA COPOLYMER MOUTHRINSE IN A THAI POPULATION

Petcharat Kraivaphan[1], Cholticha Amornchat[2] Titikan Laothumthut[3] and Terdphong Triratana[4]

[1]Department of Pharmacology, [2]Department of Microbiology, [3]Department of Oral Medicine, [4]Department of Oral Pathology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

Abstract. This investigation studied triclosan retention and plaque viability in a group of healthy human subjects from Bangkok, Thailand, 12 hours after using a mouthrinse containing a triclosan/PVM/MA copolymer system. The results show the retained triclosan in the dental plaque was with in or higher than the minimum inhibitory concentration (MIC range 0.27-6.25 μg/ml), indicating the triclosan in this product remains at an effective concentration in dental plaque. The 12-hour post-application evaluation demonstrated only 36.5% viability of oral bacteria in dental plaque after a one-time use of the mouthrinse. This study shows the benefits of using a mouthrinse containing a triclosan/PVM/MA copolymer system for providing 12 hours long-lasting anti-bacteria and dental plaque control.

INTRODUCTION

Using a toothbrush and toothpaste is recognized as routine oral hygiene for many people. Over the years, many studies have documented the efficacy of tooth brushing for its cleaning and protective benefits. Tooth brushing alone may not clean all the surfaces of the teeth, and may leave residual plaque (Frandsen, 1986). Therefore, various oral care products have been used to improve oral hygiene. Mouthrinses have been used as chemical supplements to the mechanical oral hygiene offered by toothbrushing with toothpaste (DePaola *et al*,1989; Gultz *et al*, 1998; Kaim *et al*, 1998). A mouthrinse may enhance the efficacy of tooth brushing, loosening adherent plaque and biomass (Singh *et al*, 1990; Lobene *et al*, 1992). A mouthrinse can also penetrate into the interproximal spaces difficult for toothbrushes to reach. The efficacy of mouthrinses in improving oral health has been studied over the years (Loe *et al*, 1976; Mandel, 1994). The American Dental Association (ADA) initiated requirements for testing mouthrinses that were seeking ADA approval for marketing claims (Mandel, 1994).

For the past two decades, a toothpaste developed with a patented technology containing triclosan and a polyvinylmethyl ether maleic acid (PVM/MA) copolymer has been well documented to provide multiple benefits in many clinical studies such as caries prevention, anti-plaque, prevention and treatment of gingivitis, reduction of dental

Correspondence: Assoc Prof Petcharat Kraivaphan, Department of Pharmacology, Faculty of Dentistry, Mahidol University, 6 Yothi Road, Ratchathewi, Bangkok 10400, Thailand.
Tel: 66 (0) 2203 6555 ext 6481 # 13; Fax: 66 (0) 2203 6484
E-mail: dtpvp@staff2.mahidol.ac.th

calculus and breath freshening (Gaffar *et al*, 1990,1994; Volpe *et al*, 2002; Williams and Cummins, 2003). The anti-plaque and anti-gingivitis benefits are associated with the triclosan/PVM/MA copolymer technology. This toothpaste is marketed under the brand name of Colgate® Total®. Although Colgate® Total® has been developed with several variants, its core technology, triclosan plus the PVM/MA copolymer, remains unchanged. The key antimicrobial ingredient in Colgate® Total®, triclosan, was developed by Ciba-Geigy Corporation many decades ago as a broad spectrum antimicrobial agent to be used in consumer products. It is now used in soaps, deodorants and toothpaste. Triclosan was first introduced in oral care products about two decades ago (Volpe *et al*, 2002). By itself, triclosan does not readily adhere to plaque or oral tissues and is not retained at antimicrobial concentrations for extended time periods. Research has shown the combination of triclosan and PVM/MA copolymer increases the retention of an effective concentration of triclosan in the oral cavity (Nabi *et al*, 1989; Gaffar *et al*, 1990, 1994). We previously conducted a study in a Thai population to show Colgate® Total® provides the long-lasting anti-bacterial protection after brushing (Amornchat *et al*, 2004). The results showed the concentration of triclosan detected after 12 hours was within or higher than the MIC. An effective MIC concentration leads to a reduction in bacterial viability in dental plaque, which supports long-lasting plaque control. This study confirmed the effective clinical results from long-term clinical studies (Triratana *et al*, 1993, 1994). This investigation was designed to determine the retention of triclosan in dental plaque and its antibacterial effects in a Thai population after a 12-hour single use of a mouthrinse with a triclosan/PVM/MA copolymer system, to determine whether it provides long-lasting anti-bacterial benefits against dental plaque.

MATERIALS AND METHODS

Study design

This study was a two product, double-blind randomized parallel clinical trial. It was conducted over a 16-day period with the participation of 65 healthy male and female human volunteers from Bangkok, Thailand. Subjects, meeting all inclusion and exclusion criteria, were recruited. Study inclusion criteria were: healthy male or female human volunteers 18-65 years of age (inclusive), with a minimum of 20 natural, uncrowned teeth (excluding third molars), subjects were able and willing to give informed consent and had a plaque score of at least 1.5 using the modified Quigley-Hein plaque index (Turesky *et al*, 1970). Subjects were excluded if they had a history of allergy to personal care/consumer products or their ingredients, relevant to any ingredient in the test products as determined by the dental/medical professional monitoring the study, a medical condition requiring pre-medication prior to dental procedures/visits, a medical condition precluding the subject from eating/drinking for 2 hours, and subjects with untreated dental conditions, such as advanced periodontal disease, five or more decayed, and/or untreated oral/dental lesions. A subject was also excluded if he/she wore an orthodontic appliance, presented with abnormal salivary function, required the use of drugs that affect salivary flow (*eg*, anticholinergic, adrenergic, antihistamines, vasoconstrictors/decongestants, etc), reported the use of antibiotics one month prior to or during this study, or used any over the counter medications other than analgesics (*ie*, aspirin, ibuprofen, acetaminophen, naproxyn, etc). Women who were pregnant or breastfeeding were also excluded as well as immune compromized individuals (HIV, AIDS, immuno suppressive drug therapy).

Study products

The oral rinses used in the study were: Colgate® Plax oral rinse, containing 0.03% triclosan and 1.5% PVM/MA copolymer, a product marketed in Thailand by Colgate-Palmolive (Thailand), Bangkok, Thailand, and a Colgate® Neutrafluor oral rinse, containing 0.05% sodium fluoride (no triclosan and no PVM/MA copolymer). Neutrafluor was used as a fluoride placebo control rinse in the study. Colgate® Cavity Protection, a commercial fluoride toothpaste, containing 0.83% sodium monofluorophosphate, was used as a washout product and used for routine oral care during the study period. The subjects and clinic staff were blinded to the identity of the triclosan/PVM/MA copolymer and placebo rinse bottles.

Study procedures

Soft and hard tissue exams were completed for subjects who satisfied the inclusion criteria. The washout phase started one week prior to Day 1 of the study; Colgate® Cavity Protection toothpaste and a Colgate® Plus™ Adult Soft Brush (Colgate-Palmolive, New York, NY) were provided to each subject. Study subjects were instructed to brush twice daily with the washout toothpaste until the first day of the study. Subjects were instructed to refrain from tooth brushing and the use of chewing gum, hard candy or lozenges after 8:00 PM on the evening before Day 1 of the study. Subjects were asked to refrain from oral hygiene on the mornings of their scheduled visits but they were allowed to eat or drink that morning (at least 1 hour prior to the scheduled arrival at the clinic). Subjects were randomly divided into two groups: one group (n=33) was assigned to receive the placebo rinse and the other group (n=32) was assigned to receive the triclosan/PVM/MA mouthrinse. Prior to brushing, a baseline plaque sample was obtained by a dental clinician. Under supervision, each subject then brushed the left side of his/her mouth with 1.5 grams assigned toothpaste for 45 seconds and expectorated. The subjects then rinsed with 20 ml of an assigned rinse for 60 seconds and expectorated. Subjects refrained from eating or drinking for 2 hours after brushing and rinsing and then were permitted to eat and drink as desired thereafter. No further tooth brushing, rinsing or other forms of dental hygiene were permitted over the next 12 hours. The use of chewing gum, hard candy or lozenges were not permitted on the clinic visit days. Follow-up plaque samples were collected from the right side of each subject's mouth at 6 and 12 hours after rinsing. The viability of the plaque obtained at baseline (0 hour; before brushing), 6 and 12 hours post-rinsing was determined along with the triclosan retention levels at 6 and 12 hours post-rinsing. After completion of the plaque collection for the triclosan retention phase, subjects started the second washout period, which continued for 1 week. After this washout period, subjects returned to the dental clinic for the second part of the cross over study and repeated the procedure described above with the other product.

Determination of triclosan retention in dental plaque

The dental plaque collected at different times was weighed and frozen at -20ºC before analyzing using a gas chromatographic method (GC). Briefly, calibration standards were prepared with levels of 0.01-1.5 µg/ml of triclosan. Each dental plaque sample was rehydrated, aliquoted and taken to dryness under nitrogen at ambient temperature. GC analysis was performed on 1.0 µl of reconstituted extract for triclosan concentration in µg/ml as a standard concentration used in oral care products. The data were analyzed statistically as described below.

Determination of plaque viability by fluorescent microscopy

Dental plaque samples collected during the second phase of the study were subjected to plaque viability determination as previous described (Herles *et al*, 1994; Netuschil *et al*, 1995; Amornchat *et al*, 2004). Briefly, each sample of dental plaque was placed on a regular specimen glass slide and stained with 40 µl of a dye solution containing the green fluorescent compound, 5-chloromethyfluorescein diacetate (cell Tracker™ Green CMFDA, Molecular Probes, Eugene, OR, USA) and the red fluorescent compound, ethidium homodimer 1 (EthD-1). After 15 minutes of staining, the slide was rinsed with 40 ml of buffer solution. The plaque sample was then covered with a regular microscopic specimen cover slide for evaluation under a Nikon E600 fluorescent microscope equipped with an excitation filter B-2A (excitation: 450-490 nm, emission: 520 nm) and an excitation filter G-2A (excitation: 510-560, emission : 590 nm). A total of eight fields were examined at 200 x magnification under the microscope. The ratio of dead to live bacteria in the dental plaque was determined (Table 1).

Table 1
Measurement score for dental plaque viability.

Score	Color of stain[a]	% Viability
1	All green	100
2	Green with some red	75
3	Green and red to the same extent	50
4	Red with some green	25
5	All red	0

[a]The representative images are shown in Fig 1.

Statistical analysis

For the triclosan retention study, the differences between the mean values at baseline, 6 and 12 hours post-rinsing were evaluated using a paired *t*-test. For plaque viability data analysis, a 2-way ANOVA was performed to determine the difference between the two groups.

RESULTS

Sixty-five subjects were included in the study. Sixty-one (22 males and 39 females, age ranges 19-48 years) completed all aspects of the study. No adverse effects were reported or observed at any time during the study. Subjects who did not complete the study did so for personal reasons unrelated to the clinical study.

Retention of triclosan in dental plaque phase

Six and 12 hours post-rinsing, subjects who used the triclosan/PVM/MA copolymer mouthrinse showed retention of triclosan at

Table 2
Triclosan concentration in dental plaque.

Mouthrinse	Mean concentration (± SD) µg/ml		
	Baseline	6 hours	12 hours
Plax	0.08 (0.05)[a]	8.42 (5.52)[b]	2.15 (0.99)[b]
Neutrafluor	0.20 (0.41)[a]	0.23 (0.40)[b]	0.14 (0.13)[b]

[a]No significance of ANOVA comparison of baseline means, $p>0.05$
[b]Significance of ANOVA comparison of 6 hour means, $p<0.001$

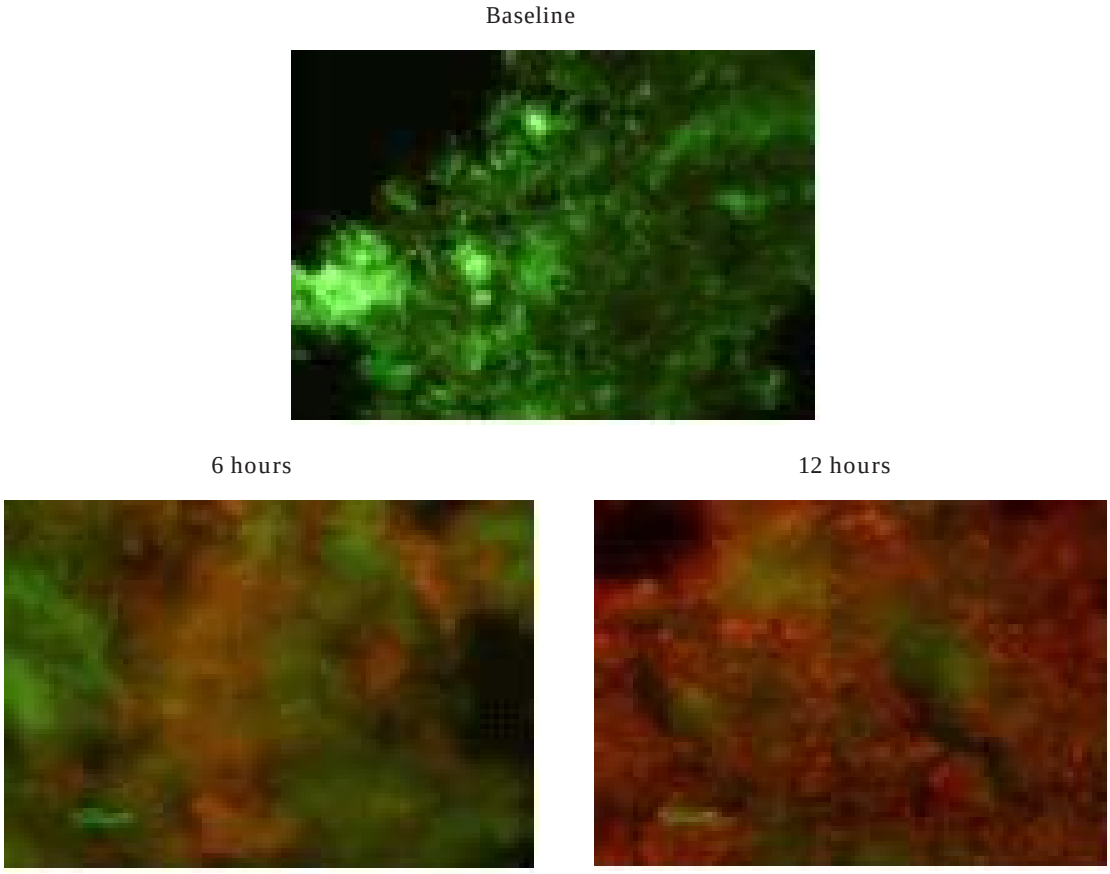

Fig 1–Representative fluorescent images of plaque viability after use of a triclosan/PVM/MA mouthrinse.

Table 3
Plaque viability score at baseline, 6 and 12 hours.

Mouthrinse	Mean score (± SD)		
	Baseline	6 hours	12 hours
Plax	1.32 (0.18)[a]	2.91 (0.35)[b]	3.54 (0.02)[b]
Neutrafluor	1.34 (0.15)[a]	1.39 (0.04)[b]	1.38 (0.08)[b]

[a]No significance of ANOVA comparison, $p>0.05$
[b]Significance of ANOVA comparison, $p<0.001$

8.42 ± 5.52 µg/ml and 2.15 ± 0.99 µg/ml, respectively. It should be noted that because of the matrix effect, occasionally samples assumed to contain no triclosan might produce very small detectable background signals. This is due to background interference as is usually seen in chromatographic procedures (*ie*, in the placebo and at time 0 in the test group). However, the group using the triclosan/PVM/MA copolymer mouthrinse

Table 4
Percent plaque viability.

Mouthrinse	Baseline	6 hours	12 hours
Plax	92.0[a]	52.0[b]	36.5[b]
Neutrafluor	90.5[a]	90.2[b]	90.5[b]

[a]No significance of ANOVA comparison, $p>0.05$
[b]Significance of ANOVA comparisons, $p<0.001$

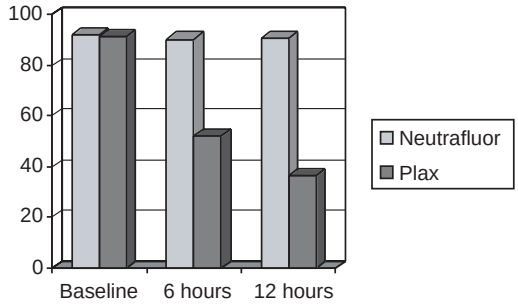

Fig 2–Plaque viability changes over the 12 hour test period. Before use of the mouthrinse, plaque viability was above 90% for both groups. More live bacteria presented at the baselines. After use of the triclosan/PVM/MA mouthrinse at 6 and 12 hours, plaque viability decreased from 92.0% to 52.0% and 36.5%, respectively, whereas the control group remained virtually unchanged.

had a significant amount of triclosan retention, which gave a much higher result than the background signal (Table 2).

Dental plaque viability

The results of the dental plaque viability at 6 and 12 hours following one-time rinsing with triclosan/PVM/MA copolymer mouthrinse *vs* the placebo rinse are shown in Tables 3 and 4, presented as viability score and percent viability. The mean values of the triclosan/PVM/MA copolymer mouthrinse were significantly different from the placebo mean values at both 6 and 12 hours post-rinsing ($p<0.001$). Table 4 and Fig 2 show the percent plaque viability at baseline and changes at 6 and 12 hours post-rinse. Before use of the mouthrinse, plaque viability ranged from 90.5% to 92.0% for both groups. After use of the triclosan/PVM/MA copolymer mouthrinse, at 6 and 12 hours, plaque viability decreased to 52.0% and 36.5%, respectively, whereas the placebo group remained virtually unchanged compared to baseline at around 90%.

DISCUSSION

An effective oral care product requires the active ingredient to be both delivered adequately in the oral cavity and to remain active for a reasonable duration after application. In the technology development process, both laboratory and clinical research are needed to confirm *in vitro* and/or *in vivo* efficacy of an ingredient or a complete formula. Of the many laboratory parameters, probably the most used for assessing antimicrobial agents is the MIC. It is a factor which reflects the effectiveness of an antimicrobial agent against representative pathogens. In addition to having effective activity at its MIC level, long term retention of the active ingredient in a formula used in the mouth is also highly desirable. This factor is also known as "substantivity". Appropriate substantivity helps define the concentration required and

dosage intervals needed to deliver a prolonged period with biological activity and hence results in effective long-lasting clinical efficacy (Stanley *et al*, 1989; Fischman and Yankell, 2004). Colgate® Total® toothpaste with its unique ingredient system ensures that triclosan remains active in the toothpaste formulation with retention in a dynamic oral environment while delivering sustained biological activity (Amornchat *et al*, 2004). Triclosan's substantivity contributes to its long-lasting clinical efficacy, based on others and our own work, there is a significant correlation between MIC value detected for triclosan extracted from clinical dental plaque and reduction in plaque viability determined from clinical dental plaque (Amornchat *et al*, 2004). The control of dental plaque is an important clinical endpoint and an important parameter for clinical evaluation. Chemotherapeutics in oral care products mainly rely on their antibacterial property, which contributes to the plaque-control clinical effect, whereas the control of dental plaque provides benefit for the prevention and/or treatment of most common oral diseases, caries and gingivitis. Since triclosan also contains an innate anti-inflammatory property (Modeer *et al*, 1996), triclosan/PVM/MA copolymer in toothpaste provides both plaque control and gingivitis control (Triratana *et al*, 1993, 1994; Volpe *et al*, 2002). Our study further confirmed it is a good approach to link both laboratory evaluation and clinical end benefits together to better understand the mode of action and to provide scientific insight into the clinical benefits (Amornchat *et al*, 2004). The current investigation used a similar test procedure to determine if the triclosan/PVM/MA copolymer ingredient system would provide the same benefits in a mouthrinse formula. With respect to the correlation between triclosan concentration in dental plaque and reported MIC values, the results showed that the mean triclosan concentrations determined at 6 and 12 hours, after a one-time rinse with the triclosan/PVM/MA copolymer mouthrinse were 8.42 μg/ml at 6 hours and 2.15 μg/ml at 12 hours. These values are higher or within the reported MIC values (0.29-6.25 μg/ml) for laboratory and clinical isolates of representative gram-positive and gram-negative bacteria (Gaffar *et al*, 1990). The retention of sufficient triclosan at a level higher or at the MIC value results in sustained antibacterial effects, which is evidenced by the results at the plaque viability study. The plaque viability study with the triclosan/PVM/MA copolymer mouthrinse exhibited only 36.5% viability of oral bacteria in dental plaque at 12 hours post-rinsing.

The overall results from this study were in agreement with previous observations 12-hour post-brushing with Colgate® Total® (Gaffar *et al*, 1990; Amornchat *et al*, 2004). The significant difference between the triclosan/PVM/MA copolymer mouthrinse and the placebo control mouthrinse for both triclosan retention and plaque viability supports the uniqueness of the technology, which delivers long-lasting plaque control in a dynamic oral environment. The result of 12 hours post-rinsing on dental plaque demonstrated clear consistency of the action of the triclosan/PVM/MA copolymer ingredient system on oral plaque. This study suggests the use of an effective mouthrinse as an adjunctive oral hygiene tool can compliment conventional oral hygiene using a toothpaste and a toothbrush.

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