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The effect of a polyhexamethylene biguanide mouthrinse compared with a triclosan rinse and a chlorhexidine rinse on bacterial counts and 4-day plaque re-growth

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Abstract

Objectives: For various clinical applications, polyhexamethylene biguanide hydrochloride (PHMB) has been used for many years as an antiseptic in medicine. Recently, a 0.04% and a 0.12% PHMB mouthwash were shown to inhibit plaque re-growth and to reduce oral bacterial counts. In this study, a 0.2% PHMB mouthrinse (A) was compared with a positive control 0.12% aqueous chlorhexidine solution (B), a commercially available 0.3% triclosan/2.0% polyvinyl methyl ether maleic acid copolymer mouthrinse (Colgate Total Plax®) (C), and a negative control placebo rinse (10% ethanol, flavour) (D).

Materials and Methods: The controlled clinical study was a double blind, randomized, four replicate cross - over design. Plaque re-growth was assessed with the Turesky et al. (1970) modification of the Quigley & Hein (1962) plaque index. The antibacterial effect was assessed by taking bacterial counts on the tooth surface (smears from the buccal surface of 16/26) and mucosa (smears from the buccal mucosa in opposite of area 16/26) after the professional prophylaxis and after the first rinse with the preparations on day 1 and prior to the clinical examination on day 5. Sixteen volunteers participated and, on day 1 of each study period were rendered plaque-free, ceased toothcleaning, and rinsed twice daily with the allocated mouthrinse. On day 5, plaque was scored and smears were collected according to the protocol. A 10-day wash-out period was carried out between each rinse evaluation. Data were analysed using ANCOVA with Bonferroni HSD adjustment for multiple comparisons (colony forming units per sample) with a significance level $\alpha = 0.05$.

Results: The 0.2% PHMB mouthrinse (A) was significantly better at inhibiting plaque than the placebo (D), but significant less effective than the 0.12% aqueous chlorhexidine solution (B). There is no significant difference between A and the 0.3% triclosan/2.0% copolymer mouthrinse (C).

Bacterial count reductions (tooth surface and mucosa) with PHMB (A) were significantly greater compared with the placebo (D) and triclosan (C), but significantly lower compared with chlorhexidine (B) (tooth surface) and equally effective compared with chlorhexidine (B) (mucosa).

Key words: clinical trail; chlorhexidine; mouthrinse; plaque; polyhexamethylene biguanide; triclosan/PVM/MA copolymer

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Conclusion: Consistent with previous studies, a PHMB mouthrinse was shown to inhibit plaque re-growth and to reduce oral bacterial counts, indicating that PHMB could be an alternative to established mouthrinses in preventive applications.

For more than three decades, plaque control through mechanical and chemical methods has been the primary preventive measure against caries, gingivitis, and periodontal disease. Because mechanical means alone, such as toothbrushing and flossing, have limitations especially in inter-proximal areas, greater interest has been shown in chemical means (Kocher et al. 2000). Therefore toothpaste additions and mouthrinses have developed into a huge market and are considered valuable adjuncts to mechanical methods of plaque control (Overholser et al. 1990).

Despite the fact that the efficacy of chlorhexidine can be impaired when it is incorporated in more complex formulas like dentifrices or mouthrinses (Rosin et al. 2001), from all investigated and useable agents, chlorhexidine has had the greatest success in plaque control and is still considered the golden standard for its potential to inhibit plaque growth (Loe and Schiott 1970, Schiott et al. 1970, Mandel 1988, Heasman & Seymour 1994). However, the local side-effects of chlorhexidine, particularly extrinsic staining and taste aberrations, have limited its long-term use in oral hygiene products (Addy 1986).

Triclosan used as a benchmark control (0.3% triclosan/2.0% copolymer – Colgate Total Plax® mouthrinse; Colgate-Palmolive Company, USA) is a diphenyl ether (bis-phenyl) derivative and is related in structure to a number of bis-phenyl polychlorinated and bis-phenyl chlorophenol compounds.

Triclosan has been extensively researched and published in over 220 articles as a broad-spectrum antibacterial/anti-microbial agent with a favourable safety profile. In the last 10 years alone, there have been more than 150 publications about its application and impact in the dentistry. Despite all shown effects, no study found triclosan equal to the effectiveness of chlorhexidine (Addy et al. 1989, Jenkins et al. 1989, 1994a, Renton-Harper et al. 1996, Arweiler et al. 2001, 2002).

Therefore there is still a search in place for an agent with the effectiveness of chlorhexidine, but without the known side-effects in the long-term use.

With that in mind Rosin et al. (2001, 2002) carried out among others plaque re-growth studies with polyhexamethylene biguanide hydrochloride (PHMB). PHMB has been used for many years as an antiseptic for various applications in medicine (Larkin et al. 1992, Wagner 1995, Willenegger et al. 1995, Kramer & Behrens-Baumann 1997). In contrast to chlorhexidine (bisbiguanide), PHMB is a polymeric biguanide and has no terminal chlorbenzene substitutions (Davies et al. 1968, Davies & Field 1969). It has broad spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria and *Candida albicans* (Werner 1992).

In the studies by Rosin et al. (2001, 2002), a significant inhibition of plaque re-growth was demonstrated for 0.04% and 0.12% PHMB mouthrinses, finding no statistically significant differences to each researched commercially available mouthrinses, used as benchmark controls. Both PHMB mouthrinses, however, were less effective than the 0.12% chlorhexidine positive control rinse.

Based on these results and the PHMBs comfortable margin of safety for increasing concentration (Kallenberger et al. 1991, Kramer et al. 1993, Kühl et al. 1994, Kramer et al. 1998), the purpose of the present investigation was to compare PHMB mouthrinse in a higher concentration (0.2%) to a chlorhexidine mouthrinse as positive control and to a placebo mouthrinse as a negative control evaluating their effect on plaque re-growth and oral bacterial counts. For an additional useful comparison, the study includes a commercially available product containing triclosan.

Materials and Methods

Plaque re-growth study

This clinical study used a double-blind, four-treatment, randomized, cross-over design in a 4-day plaque re-growth model (Addy et al. 1983). The following preparations were tested:

(A) 0.2% PHMB mouthrinse: 1.0% Lavasept® containing 20% PHMB (Fresenius Kabi, Bad Homburg, Germany), 0.1% aromatic oil (Henkel, Düsseldorf, Germany), 0.1% Cremophor (Henkel), 10.4% ethanol (96%), 88.6% Ringer's solution.

(B) 0.12% Chlorhexidine mouthrinse: 6% solution of the 20% chlorhexidine digluconate stock solution (Henkel), 94% deionized water.

(C) 0.3% Triclosan/2.0% copolymer mouthrinse: Commercially available Colgate Total Plax® mouthrinse (Colgate-Palmolive Company, New York, NY, USA) containing 0.3% 2,4,4′-Trichloro-2′-hydroxydiphenyl ether/2.0% polyvinyl methyl ether maleic acid (PVM/MA) copolymer.

(D) Placebo mouthrinse: 0.1% aromatic oil (Henkel), 0.1% Cremophor (Henkel), 10.4% ethanol (96%), 89.4% deionized water.

Mouthrinses A–D were prepared by the pharmacy of Greifswald University and all rinses were labelled to identify the subject and study period. Individually sealed code breakers for the subjects and products were kept by the supervisor of the study (A. K.).

Sixteen subjects (six male and 10 female students from the Greifswald Dental School, mean age 21.1 years) volunteered to participate in the study. Subjects had a documented high standard of oral hygiene and gingival health and conformed to the following criteria:

- a minimum of 25 scorable teeth;
- no more than one full-coverage restoration;
- not wearing intra-oral appliances;
- good general health without medical or pharmacological histories, which might compromise the validity of the study.

Screening and selection of participants was carried out by a single investigator (G. S.-M.), who also scored all visits of the trial. The protocol for the study was approved by the Ethics Committee of the Medical School of Greifswald University, and all participants gave verbal and written informed consent to participate.

On day 1 of each of the four study periods (Fig. 1), the participants received an oral soft- and hard-tissue examination and a professional scaling and polishing to remove all visible calculus, plaque and extrinsic tooth stain. After

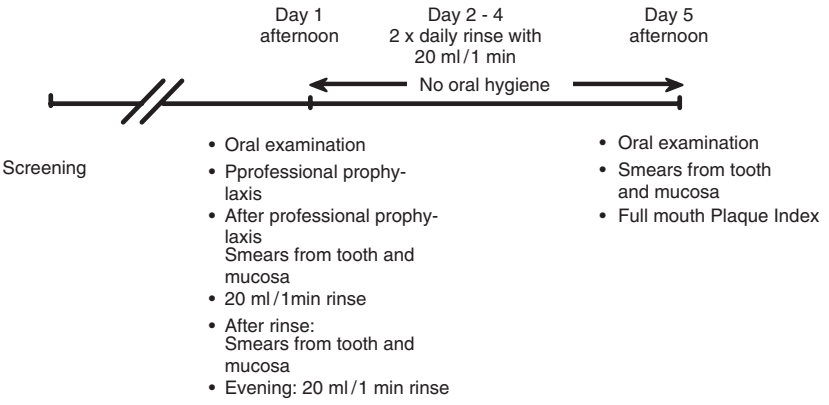

Fig. 1. Outline of the clinical trial.

30 s of rinsing with 30 ml of tap water, the first smear was taken from the tooth and mucosa for the determination of the baseline bacterial counts. These data were entered as co-variate into the determination of the bacterial count reduction by the 20 ml of the assigned mouthrinses, after rinsing for 60 s. The mucosal smears were taken from the buccal mucosa opposite areas 16 (baseline) and 26, respectively. The tooth smears were taken from the buccal surface of 16 (baseline) and 26, respectively.

On subsequent days, the participants suspended normal oral hygiene habits and rinsed twice per day, after breakfast and in the evening, with 20 ml of the allocated mouthrinse for 60 s. The last rinse was performed in the morning of day 5. Compliance was assessed by measuring the residual mouthwash in the bottles. The clinical evaluation took place between 15:00 and 17:00 hours on day 5 (Fig. 1). Following an oral soft- and hard-tissue examination, a tooth and mucosal smear were collected.

The accumulated plaque was disclosed using a disclosing solution (MIRA-2-TON, Hager & Werken, Duisburg, Germany) and scored using the Turesky et al. (1970) modification of the Quigley & Hein (1962) plaque index (QHI). The scores were taken at six surfaces per tooth: mesio-, mid-, and disto-buccal, and mesio-, mid- and disto-lingual. All teeth except 3rd molars were examined. After the assessments, each participant received a rubber-cup polishing to remove all plaque. A total of four experimental periods were repeated until all subjects had used the four different mouthrinse preparations. Each test cycle was followed by a 10 days wash-out period, in which the subjects resumed their normal oral hygiene habits.

Reproducibility of clinical examinations

The clinical investigator (G. S.-M.) was given intensive clinical training to achieve a high reproducibility of the QHI scorings. Prior to the study, five calibrating sessions were performed in which the study investigator was calibrated against a very experienced investigator (T. K.). Each time, two patients were examined, during which a third clinician (A. W.) performed the examinations while the other two investigators watched and scored independently. In the last calibration session before the start of the study, an inter-examiner reliability of $\kappa \geqslant 0.78$ was achieved for the QHI. Before the start of each cell of the trial, one additional session was performed to check and maintain consistency in the plaque scorings of the clinical investigator. The respective κ values for the sessions prior to the other three cells of the trial were 0.79, 0.80, and 0.77.

Bacterial count measurements

The antibacterial effects of the mouthrinses were assessed by measuring bacterial counts on the tooth surface (smears from the buccal surface of 16 (baseline) and 26) and on the mucosa (smears from the buccal mucosa opposite areas 16 (baseline) and 26 above the secretory duct of the *Gl. parotis*), respectively. The smears were taken on days 1 and 5 (see above Fig. 1). To take the smear from the tooth surface, the buccal surfaces of the teeth were dried with a gentle stream of air before the smear was taken with a SL 1/2 curette (Hu–Friedy Manufacturing Company Inc., Chicago, IL, USA), moving from cervical to occlusal. The mucosal smears from the mucosa were taken using sterile cotton wool swab sticks ''Uno'' (Maersk Medical A/S, Lynge, Denmark). The curettes and swabs (samples) were placed in a casein-pepsin-solution and agitated for 60 s for taking bacterial counts. The solution was then serially diluted to 10^{-1}, 10^{-2}, and 10^{-3} (phosphate buffered saline) and added to casein–peptone–Agar plates. The plates were then incubated under aerobic conditions for a period of 48 h at 37°C before the colony-forming units (cfu) were counted. Results were expressed as cfu per sample.

Statistical methods

Analysis of variance (ANOVA) was used. The treatment was adjusted by period and subject (and baseline bacterial count for ANCOVA-model). Bacterial counts (cfu/sample) were positively skewed and required log transformation to fit a normal Gaussian model. For the analysis of the values after rinses of the allocated mouthrinse on day 1 and on day 5, the baseline values were included as co-variate.

In the 1st step, data were analysed using ANCOVA for simultaneous comparisons. If the test concerning the treatment groups was statistically significant, in the 2nd step, the specific differences among the means of the treatments were determined. For these comparisons the Bonferroni adjustment as an adequate multiple-comparison technique for ANCOVA was used (significance level $\alpha = 0.05$).

Results

Compliance

The amounts of mouthrinses used indicated good compliance by the participants. No adverse events or side-effects were reported or observed. Subject complaints of unpleasant taste were recorded three times for PHMB and twice for the placebo and once for chlorhexidine.

Plaque re-growth study

The plaque index data for each treatment are shown in Fig. 2. Comparisons of pairs of treatments indicated that PHMB mouthrinse (A) was significantly more effective at inhibiting plaque than the placebo (D), but significantly less effective than chlorhexidine (B). However, between PHMB and triclosan (C), and triclosan and placebo there were no significant differences, respectively (Fig. 2).

Bacterial count measurements

The results of the bacterial count measurements on the tooth surface and on the mucosa for each treatment and for the two time points investigated are presented in Figs 3 and 4.

On the clean tooth surface, after cleaning and polishing taken as baseline (co-variate) for the determination of the reducing bacterial counts by the first single rinse as expected there was no significant difference between the participants of each period.

After a single rinse with the respective treatments for 60 s directly after cleaning and polishing there was no difference between PHMB, Triclosan, and the placebo, while the aqueous chlorhexidine rinse was significantly more effective in reducing bacterial counts than the placebo rinse (Table 1).

Eight hours after the last rinse on day 5, PHMB inhibited the bacterial growth on tooth surfaces significantly more effectively than the placebo and triclosan, but significantly less compared with chlorhexidine (Table 1).

On the mucosa, for the baseline after cleaning and polishing as expected there was no significant difference between the participants in each period. After a single rinse with the respective treatments for 60 s, PHMB, triclosan, and the aqueous chlorhexidine were significantly more effective in reducing bacterial counts than the placebo rinse (Table 2). Between the PHMB, triclosan, and the aqueous chlorhexidine there were no differences (Table 2).

Eight hours after the last rinse on day 5 the bacterial colonization on mucosa was inhibited to a significantly greater degree with PHMB than with both the placebo and triclosan (Table 2). Furthermore, PHMB was equally effective as chlorhexidine (Table 2). There was no difference in bacterial count reductions between triclosan and placebo (Table 2).

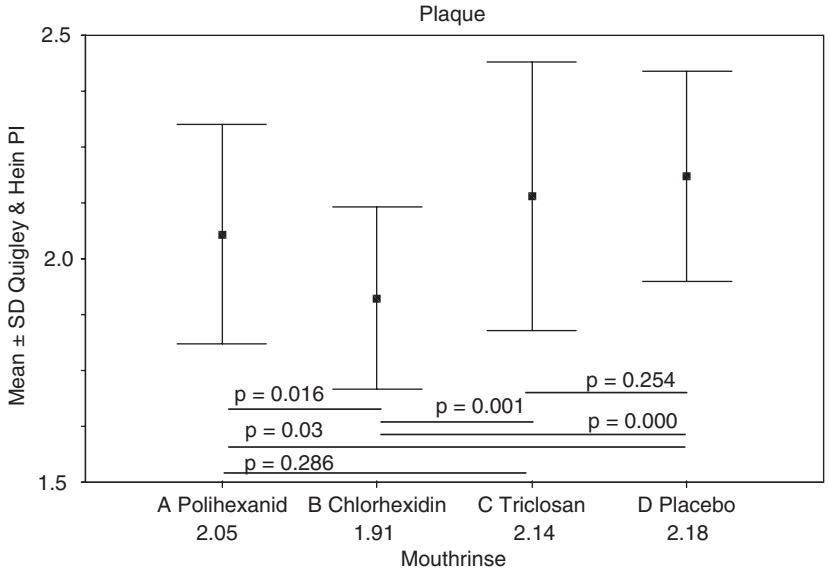

Fig. 2. Four-day plaque re-growth study: means, estimated 95% confidence intervals, and results of ANOVA for four treatments.

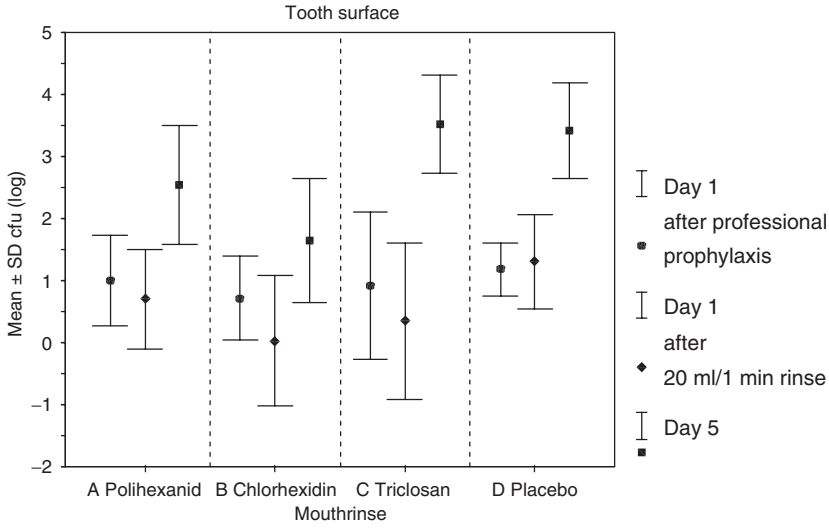

Fig. 3. Bacterial counts (colony forming units per sample, log transformed) on the tooth surface: means, estimated 95% confidence intervals, and results of ANCOVA (adjustment for multiple comparisons) for four treatments.

Discussion

In recent studies (Rosin et al. 2001, 2002), 0.04% and 0.12% PHMB rinses, respectively, were significantly more effective at inhibiting plaque re-growth than the negative control (placebo) and significantly less effective than the positive control (0.12% chlorhexidine). The results of these studies were particularly encouraging, since no differences in the plaque inhibiting effect was observed between PHMB and the included benchmark controls, a commercially available chlorhexidine rinse (Skinsept® mucosa, Henkel) diluted to the same chlorhexidine concentration as the positive control aqueous solution (Rosin et al. 2001) and commercially available mouthrinse containing essential oils (Listerine®, Warner-Lambert, Consumer Healthcare Products, Freiburg, Germany), respectively. The present study was designed to assess the plaque inhibitory effects of an increased PHMB concentration (0.2%) and to position this formulation against a negative and a positive as well as a benchmark control. The triclosan mouthrinse (C) was chosen as the benchmark control because its active agent concept (0.3% triclosan/2.0% copolymer) is one of the most evaluated oral health care products. The effectiveness of mouthrinses containing triclosan has been evaluated in 50 publications including: Abello et al. (1990), Singh et al. (1990), Williams et al. (1990), Hunter et al. (1994), Jenkins et al. (1994b), Kjaerheim et al. (1995), Schaeken et al. (1996), Moran et al. (1997), Cronin et al. (1997), Gautier et al. (2000), Arweiler et al. (2002), Shapiro et al. (2002), Yates et al. (2002).

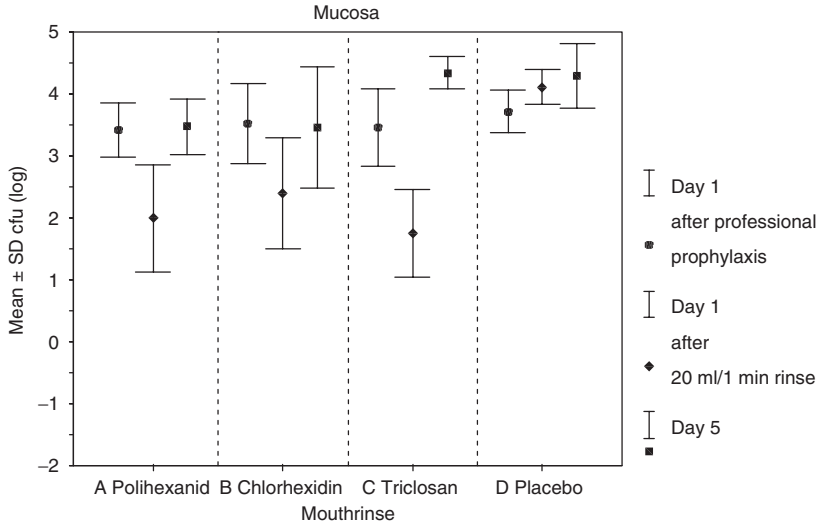

Fig. 4. Bacterial counts (colony forming units per sample, log transformed) on the mucosa: means, estimated 95% confidence intervals, and results of ANCOVA (adjustment for multiple comparisons) for four treatments.

Table 1. Results (*p*-values) based on the paired comparisons of mean bacterial counts of the treatments on the tooth surface, resulting from the ANCOVA*

	Day 5†			
	PHMB	Chlorhexidine	Triclosan	Placebo
Day 1‡ after rinse				
PHMB		0.029	0.015	0.023
Chlorhexidine	0.623		0.000	0.000
Triclosan	1.000	1.000		1.000
Placebo	0.670	0.016	0.078	

Bacterial counts after first rinse on day 1 (lower left panel – below the diagonal) and on day 5 (upper right panel).
*Bonferroni adjustment as a multiple-comparison technique for ANCOVA was used (significance level $\alpha = 0.05$).
†Adjusted $R^2 = 0.487$.
‡Adjusted $R^2 = 0.285$.

Table 2. Results (*p*-values) based on the paired comparisons of mean bacterial counts of the treatments on the mucosa, resulting from the ANCOVA*

	Day 5†			
	PHMB	Chlorhexidine	Triclosan	Placebo
Day 1‡ after rinse				
PHMB		1.000	0.002	0.003
Chlorhexidine	0.738		0.002	0.002
Triclosan	1.000	0.061		1.000
Placebo	0.000	0.000	0.000	

Bacterial counts after first rinse on day 1 (lower left panel – below the diagonal) and on day 5 (upper right panel).
*Bonferroni adjustment as a multiple-comparison technique for ANCOVA was used (significance level $\alpha = 0.05$).
†Adjusted $R^2 = 0.350$.
‡Adjusted $R^2 = 0.743$.

As in the previous studies (Rosin et al. 2001, 2002), PHMB was significantly more effective at inhibiting plaque than placebo, but lower than chlorhexidine. Between PHMB and the commercial available control mouthrinse Colgate Total Plax, there was no significant difference in its plaque inhibition at day 5. Unlike PHMB, there was no statistically significant difference between triclosan/copolymer and placebo in this study. This is, however, in contrast to the plaque re-growth study by Binney et al. (1995), where a superior plaque inhibitory activity of triclosan compared with a placebo has been demonstrated.

The plaque control depends not only on the bactericidal and bacteriostatic effects of the agent but also on its substantivity. A number of studies (Flotra et al. 1972, Loe et al. 1976, Addy et al. 1997, Yates et al. 1997) corroborate with that line of argument from Kornman (1986) who stated that substantive antimicrobial agents are, as a rule, the most effective in plaque inhibition.

To learn more about the substantivity profile of the PHMB, the bacterial count reductions were measured at three time points. The samples were collected as in the previous studies (Rosin et al. 2001, 2002): from the tooth and the mucosa; the surfaces where the bacteria actually grow (MacFarlane 1988).

After a single rinse for 60 s only chlorhexidine was significantly more effective in reducing bacterial counts than the placebo rinse on the clean tooth surface. There was no significant difference between chlorhexidine, PHMB, and triclosan rinses. However, it has to be said these data reflect likely more saliva contamination than the pellicle, containing bacteria, because the teeth were practically sterilized by polishing with rubber cups and a polishing paste in the prophylaxis session.

The sample from the tooth surface taken 8 h after last rinse on day 5 may also be problematic in terms of interpretation. This sample is assumed to reflect the number of vital plaque bacteria about 8 h after the last rinse, thus giving some information about the substantivity of the antiseptic agents tested in the mature plaque.

PHMB inhibited the bacterial growth on tooth surfaces (mature plaque) significantly more effectively than the placebo and triclosan, but significantly less compared with chlorhexidine. Although Gilbert & Williams (1987) in their pharmacokinetic study showed that triclosan even without copolymer is present in mature plaque for at least 8 h after dosage, in contrast to PHMB, triclosan showed no effects on a mature plaque about 8 h after the rinse. These results support the connection between the substantivity of an agent and plaque

inhibition (Elworthy et al. 1996) and the results of the study by Rosin et al. (2002). In the latter study, PHMB was found to be more effective than Placebo on day 5 in its bacterial count reduction despite its lower concentration (0.12%) and 12 h after the last mouthrinse. On the mucosa directly after a single rinse for 60 s, PHMB, triclosan, and the aqueous chlorhexidine rinses were significantly more effective in reducing bacterial counts than the placebo rinse. Between the PHMB, triclosan, and the aqueous chlorhexidine there were no differences. This suggests an initial equally effective bactericidal effect like chlorhexidine and triclosan.

Furthermore these results confirm the previous studies by Rosin et al. (2001, 2002) indirectly, where even after 4 h and with less concentration (0.04% and 0.12%) PHMB was statistically significantly better than placebo.

On day 5, 8 h after the last rinses, PHMB was not only more effective than placebo, but also there was no difference between chlorhexidine and PHMB. Moreover PHMB was significantly more effective than the commercially available triclosan mouthrinse, which was not significantly different to the placebo.

Thus, the results of the bacterial count reduction on day 5 about 8 h after the last rinsing both on the tooth surface and also on the mucosa indicate a higher antibacterial substantivity than triclosan.

In summary, the 2.0% PHMB mouthrinse was significantly less effective in inhibiting plaque re-growth than the positive control 0.12% aqueous chlorhexidine solution. However, because of the relatively low toxicity and good tissue compatibility of PHMB compared with chlorhexidine (Kallenberger et al. 1991, Kramer et al. 1993, 1998, Kühl et al. 1994) applications of PHMB at even higher concentration are possible. Alternatively, it would appear promising to combine PHMB with other agents and to test for synergistic antiplaque effects.

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