Transient reduction of Streptococcus mutans interdentally by chlorhexidine gel

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Abstract – Chlorhexidine gel was applied interproximally with the intent to reduce Streptococcus mutans at these sites. Bacterial samples were obtained using toothpicks, which were inserted in each interproximal space and then immediately pressed against agar plates, selective for S. mutans. Duplicate bacterial samples of non-treated subjects showed that this method gave reproducible results. Using a split mouth technique, 10 subjects were exposed to short term chlorhexidine exposures of varied intensity. At the baseline sampling about 90% of the sites showed growth of S. mutans. One week after the chlorhexidine applications about 55% were infected. After 40 d S. mutans were back to about baseline levels except for the most intensively treated interproximals, which showed 75% infected sites. Thus, at many sites the reduction of S. mutans was only transient. Four subjects with more than 1 million S. mutans per ml saliva participated in a study where salivary and interproximal levels of S. mutans were compared after a rinsing period with chlorhexidine lasting for 2 weeks. The effect of the rinses varied individually, but it was noted that several interproximal spaces could be infected even if the saliva numbers did not reach detectable levels of S. mutans.

Key words: antimicrobial treatment; chlorhexidine; Streptococcus mutans.

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The topical antimicrobial agent chlorhexidine is sometimes used to suppress Streptococcus mutans in the oral cavity (for review see 1). A consideration which makes this agent particularly interesting is that S. mutans is more sensitive to this bisbiguanide than are many other species, for example S. sanguis (2), an organism which also colonizes the teeth but with less cariogenic potential. However, successful prolonged use of chlorhexidine, either as a rinse or as a gel applied topically with mouth trays, depends on the cooperation of the patient. Recently, MALTZ-TURKIENICZ *et al.* (3) have reported that repeated short term exposures increase the bactericidal effect of chlorhexidine in vitro. A follow-up clinical study to test this approach showed that the number of S. mutans in saliva could be greatly reduced by repeated applications (4). In the present study, we have tested the effect of chlorhexidine, applied in a similar way, but monitored its effect on S. mutans in plaque samples collected from approximal tooth surfaces. These surfaces often harbor S. mutans in high numbers (5, 6) and can also be regarded as caries key-risk surfaces. As many tooth surfaces per subject were involved,

we also found it necessary to develop a new bacterial sampling method to facilitate both determination of baseline levels of infection and posttreatment monitoring of chlorhexidine's effectiveness in reducing S. mutans' challenge.

Material and methods

BACTERIAL SAMPLING METHOD

Eleven subjects participated in this part of the study. Seven were selected among the staff and four among the patients at the Department of Cariology, University of Lund. After isolating areas of the dentition using cotton rolls and drying with compressed air, a wooden, triangular toothpick (Te Pe blå, Eklund and Petersson AB, Malmö, Sweden) was pressed into each interproximal space until firm contact was obtained with both teeth. If the space was too wide, the toothpick was first pressed against one tooth and then against the other. Both sides of the toothpick were then immediately pressed against selective mitis salivarius bacitracin (MSB) agar (7) in contact Petri dishes having the convex agar surface elevated over their top edges (Nunc, Roskilde, Denmark). After anaerobic incubation in 95% N_2, 5% CO_2 for 48 h, the numbers of S. mutans colony forming units (CFU) of each pair of impressions were counted under a binocular microscope. The sum of the two was considered the degree of infection in that interproximal space.

Up to 100 CFU on each single impression were usually easily counted. Each interproximal space was then placed in any of four groups: 0 for undetectable levels, 1–20, 21–100 or >100 for the highly infected sites.

The whole procedure was repeated 2–7 d after the first sampling occasion.

TREATMENT WITH CHLORHEXIDINE GEL

Ten adult subjects, patients at the Department of Cariology, participated in the study. They were selected from a group whose routine bacteriological samples had shown S. mutans in saliva exceeding 1 million CFU/ml. During the treatment period, none of the subjects had open carious lesions, but three of them had temporary fillings. All interproximal spaces were sampled and treated (see below) but in the evaluation of the effect, 19 spaces with temporary fillings and the spaces between the central incisors were excluded.

After baseline sampling with toothpicks as described above, all teeth were cleaned with rubber cups and pumice. The interproximals were either flossed or cleaned with a toothpick.

Using a split mouth technique, three quadrants were treated with chlorhexidine as follows: One quadrant was treated three times for 3 min (3 × 3) with a 10 min interval between each treatment. Another quadrant was treated 2 × 3 min with a 10 min interval and a third quadrant was treated once in 3 min. The fourth quadrant was left untreated to serve as a control.

The chlorhexidine gel (Hibitane Gel® 1%, ICI, England) was applied using a facial approach in each interproximal space with a syringe after the teeth had been dried with compressed air and the area isolated with cotton rolls. After 3 min, the gel was removed using a stream of water and suction. Finally, the patient rinsed with water. The whole procedure was repeated after 2 d. At this time, however, no cleaning of the teeth was performed.

Bacterial samples were taken immediately before the second chlorhexidine treatment (day 2) and then 7 and 40 d after the second chlorhexidine treatment.

CHLORHEXIDINE RINSING TEST

To determine how the numbers of S. mutans detected by the new sampling method were affected by conventional chlorhexidine rinses, a group of four subjects was selected. Baseline toothpick samples were collected before a rinsing period of 14 d started. The samples were then repeated at days 2, 9 and 39–45 after the termination of the rinses. At each occasion, a paraffin-stimulated saliva sample was also collected. The saliva was transferred to VMG II transport medium (8) and the number of S. mutans per ml determined (9).

For the rinses, Hibitane Dental® 0.2% (ICI, England) was used. The patients were instructed to use 10 ml for 1 min twice each day during the 2-week period.

Results

SAMPLING METHOD

A total of 259 interproximal spaces were sampled for S. mutans. Of these, 99 (38%) had more than 100 CFU. S. mutans was not detected in 83 (32%). When the sampling was repeated 2–7 d

Table 1

Comparison between number of colony forming units (CFU) of S. mutans obtained from 259 interproximal sites at two different occasions

First sample No. of CFU	n	Second sample >100	21–100	1–20	0
>100	99	80*	14	5	0
21–100	28	6	14	7	1
1–20	49	1	4	30	14
0	83	1	2	19	61
	$n=259$				

* Figures indicate number of interproximal sites.

later, 80 still had more than 100 CFU while 61 were negative. Table 1 shows that only 10 samples showed a change greater than one class.

CHLORHEXIDINE GEL TREATMENT

The total number of interproximal spaces exposed to each of the local chlorhexidine treatments varied from 51 to 55 (Table 2). At the baseline sampling, about 90% of these were infected by S. mutans. Two days after the first chlorhexidine application, the number of infected sites had decreased (53–66%). A decrease to 78% was observed in the control group. One week after the second treatment, the situation was about the same as after the first. After 40 d, however, the control quadrants and the quadrants treated 1 or 2 × 3 min were back to about original values while in the 3 × 3 min quadrant 75% of the sites were infected.

At the baseline examination, 100 approximal sites showed >100 CFU S. mutans. The reductions of S. mutans at these particular sites are illustrated in Fig. 1. It appears that the best 1-week effect was obtained after the 3 × 3 min treatment. Here, only 3% showed high S. mutans values, >100 CFU, compared with, for example, 26% in the 1 × 3 group. After 40 d, negative sites could only by found among the most intensively treated sites. The number of sites with >100 CFU was about the same, 52–65%, irrespective of treatment. A detailed analysis was also performed for sites which had only 1–20 CFU at the baseline registration. Eight sites were treated 3 × 3 min. After 7 d, seven were free of S. mutans; after 40 d, five of them were still negative. Similar results were obtained with the 2 × 3 min treatment. If only exposed for one gel application, four out of 13 sites were negative after 40 d. In the control group, three of the original 15 sites with 1–20 CFU were negative after 40 d.

Table 2

No. of infected interproximal sites after various chlorhexidine treatments

No. of chlorhexidine applications at each occasion*	Total no. of sites	% infected sites			
			Days after first treatment		
		Baseline	2	9	42
3	51	90	55	49	75
2	53	91	66	64	85
1	55	82	53	51	76
0	55	87	78	76	87

* Applications were repeated day 2 immediately after sampling.

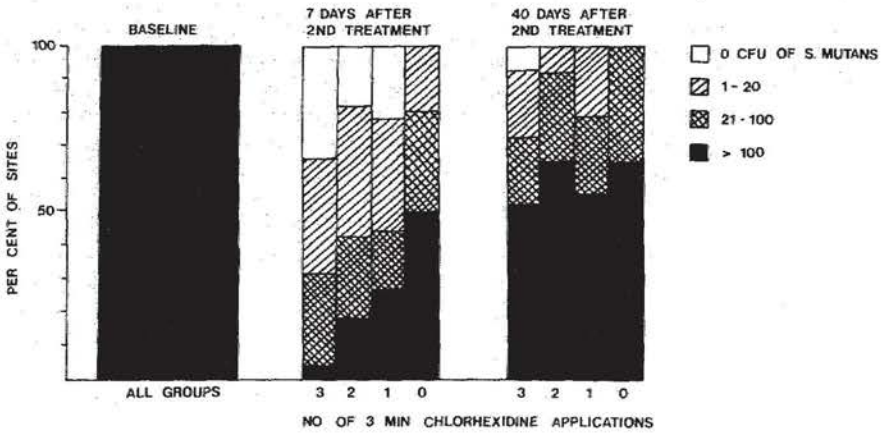

Fig. 1. Effect of chlorhexidine gel on interdental S. mutans in interproximals with > 100 CFU at baseline. Number of sites in each group: 29 (3), 27 (2), 23 (1) and 21 (0 applications).

EFFECT OF RINSING WITH CHLORHEXIDINE

The saliva of two subjects, CS and US, showed zero S. mutans 2 d and 1 week after the termination of the chlorhexidine rinses (Table 3). However, a few sites in each of these subjects were still positive for S. mutans. In one of the two subjects (CS), the situation after 1 month was practically identical to baseline. In the other, the CFU in saliva post-treatment were only 0.08×10^6 compared to 5.7×10^6/ml at the baseline. Sixteen sites were now infected compared to 23 at baseline.

In the two other patients, the effect of the rinses was not as evident. In subject AL, S.

Table 3

Effect of chlorhexidine rinses on S. mutans

		Sampling occasions			
			Days after termination of chlorhexidine rinses		
Subject	No. of:	Baseline	2	9	39–45
CS	Infected sites of total	25/26*	4/26	2/26	26/26
	CFU in saliva/ml	1.2×10^6	0	0	1.0×10^5
US	Infected sites	23/25	7/25	16/25	16/25
	CFU in saliva	5.7×10^6	0	0	0.08×10^6
LS	Infected sites	25/26	11/26	19/26	20/26
	CFU in saliva	1.5×10^6	0.69×10^6	0.26×10^6	0.46×10^5
AL	Infected sites	24/24	23/25	24/24	–
	CFU in saliva	1.4×10^6	0.64×10^6	10.7×10^5	–

* No. of sites infected/total number sampled.

mutans was not eliminated from the interproximal spaces although a reduction in saliva was observed at the first post treatment examination (0.64×10^6 CFU) compared to the baseline value (1.4×10^6 per ml). The fourth subject, LS, had 20 sites with S. mutans after 1 month compared to 25 at baseline.

Discussion

This study is based on two concepts. First, it is assumed that S. mutans is a cariogenic microorganism and that the removal of this bacterium would have a caries protective effect. HAMADA & SLADE's review of much of the pertinent literature and their own conclusions (10) support this view, although we are aware of no studies in which S. mutans has been totally eliminated as the single factor with a concomitant reduction of caries in humans. The second concept is that we feel that there is a need for anticaries treatment based on the alteration of dental plaque's composition, but one which does not rely solely on the patient. It would certainly be an advantage if a patient could leave the dental office with a less cariogenic microflora following a professionally administered antimicrobial treatment.

Chlorhexidine has been widely used in dentistry and seems to be a logical starting point for such efforts (11). Our results confirm the overall view that chlorhexidine is effective against S. mutans. There is also some support for the observation by MALTZ *et al.* (3) that repeated short term exposures are more effective than single applications. On the other hand, our results after 40 d cannot be regarded as satisfactory from a clinical point of view. Most sites showed regrowth of S. mutans with many sites reaching the original quantitative levels of infection.

MALTZ *et al.* (4) used saliva samples to monitor therapy, while in this study each interproximal space was sampled. Our method is based on the fact that S. mutans has a very localized intraoral distribution and does not readily spread from one tooth to another (12). This approach also lends itself to testing by a controlled split mouth technique. Although there certainly was a risk of reinfection from the non-treated side or from the mucosal surfaces, the data suggest that most surfaces were never totally free of S. mutans, at least not those heavily infected from the beginning.

Among the patients rinsing with chlorhexidine for 14 d, only one (CS) showed a pronounced reduction in the number of infected sites after 9 d. This subject had two sites with S. mutans in contrast to 25 at baseline, but 1 month later original values were recorded again. Subject US presents an example of a saliva sample yielding zero S. mutans detectable while 16 interproximal spaces out of 25 still harbored this bacterium. This observation might indicate that a posttreatment saliva sample may actually overestimate the effect of chlorhexidine. Other surfaces than the approximals probably contribute to the salivary pool of S. mutans especially occlusals. These retentive surfaces may be more easily affected by antibacterial agents as found in JORDAN & DE PAOLA's studies using topical vancomycin gel treatments (13).

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