Mouth-rinsing with chlorhexidine causes a delayed, temporary increase in the levels of oral viridans streptococci

Lauri H. Vaahtoniemi, Kai Karlqvist, Mikko Altonen and Simo Räisänen
Department of Oral and Maxillofacial Surgery, Institute of Dentistry, University of Oulu, Oulu; Kokkola Health Center, and Clinical Laboratory, Central Hospital of Keskipohjanmaa, Kokkola; Finland

Vaahtoniemi LH, Karlqvist K, Altonen M, Räisänen S. Mouth-rinsing with chlorhexidine causes a delayed, temporary increase in the levels of oral viridans streptococci. Acta Odontol Scand 1995;53:226–229. Oslo. ISSN 0001–6357.

The indigenous oral flora of 27 volunteers was monitored longitudinally over a 4-week period. Bacteria attached on buccal epithelial cells were counted by microscopy. Salivary bacterial colonies and the presence of alpha-hemolysis were examined after aerobic culturing on blood agar plates. The buccal and salivary bacterial counts were stably maintained in most subjects in the two repeated base-line samplings taken at 1-week intervals. Rinsing with a chlorhexidine mouthwash 45 min before sampling dramatically reduced the amount of epithelial cell-adherent bacteria. One day after the chlorhexidine rinse, however, the numbers of the epithelial cell-adherent bacteria exceeded the base-line level, and a similar decrease–increase pattern of changes was detected for the salivary alpha-hemolytic streptococcal counts. The non-hemolytic salivary bacterial counts were not affected by chlorhexidine. Subsequent weekly samplings showed no difference from the base-line samplings. The chlorhexidine-induced, delayed increase of viridans streptococci on oral epithelial surfaces should be considered a possible risk factor in medically compromised patients. □ *Anti-infective agents; mouth mucosa; oral hygiene*

Lauri H. Vaahtoniemi, Health Center of Kokkola, Mariankatu 28, FIN-67200 Kokkola, Finland

Chlorhexidine is regarded as an outstanding oral antiseptic agent. Numerous studies have appraised its power to eliminate the build-up of bacterial accumulations in the oral cavity, yet the side effects of this product are considered innocuous (1). During the past 2 decades chlorhexidine has gained widespread usage as a preoperative decontaminant in oral surgical operations and as an adjunct to oral hygiene regimens in dental plaque-related diseases.

Chlorhexidine is retained on oral mucosal surfaces. We recently introduced a novel method for measuring changes in the magnitude of oral mucosa-adherent microflora and reported that a single rinse with chlorhexidine caused, after an initial, sharp decrease, a profound and long-lasting increase of the oral mucosa-associated streptococci-like bacteria in volunteers (2). Surprisingly, considering the widespread usage of chlorhexidine, there are only a few studies in which, after a single chlorhexidine rinse, salivary or dental plaque bacterial counts have been monitored for longer than a few hours only. Consequently, relatively little interest has been paid to the oral microecologic turmoil following the haphazard use of chlorhexidine products.

The bacterial species generally found on the smooth oral epithelial surfaces are mainly considered to be viridans streptococci (3). The term 'viridans' ('greening') has been assigned to streptococci with the ability to form a green-colored alpha-hemolysis around colonies grown on blood agar. The viridans streptococci are generally considered harmless commensals of the oral cavity, or even beneficial since they provide colonization resistance against pathogens in the oral cavity (4, 5). Nevertheless, *Streptococcus mitis*, which is possibly the most prevalent oral mucosa-associated alpha-hemolytic streptococcus (6), seems to have potential for life-threatening infections in patients receiving aggressive antineoplastic chemotherapy (7 ,8). In fact, the occurrence of *S. mitis* sepsis has been on the increase during the past decade (8). Further, *S. sanguis* is possibly the most frequent cause of bacterial endocarditis (9).

The aim of this study was to elucidate the effects of a single rinse of chlorhexidine on the salivary aerobic bacterial counts and their relation to the oral mucosal epithelial cell-adherent microflora.

Materials and methods

The study population

The study population comprised 27 dental students (4 men, 23 women) whose age ranged between 19 and 31 years. All participants gave their signed, informed consent to participate in this study; the study protocol had been approved by the ethical committee of the Medical Faculty, University of Oulu. The subjects were advised to maintain a twice daily (morning and evening) toothbrushing regimen during the study period, except for the mornings before sampling, when no toothbrushing was allowed. The participants were requested not to eat or drink for at least 1 h before the sampling.

During the course of the study the use of mouthrinses or lozenges with suggested antimicrobial properties was not allowed, nor was it allowed to change one's regular toothpaste brand. The use of antibiotics and other drugs during the study period was asked about on a questionnaire. The continuous use of prescribed drugs (for example, contraceptive drugs) during the entire study period was accepted. The last two samplings from two female participants had to be excluded from the study because of starting of antibacterial therapies.

The study design

Altogether six samples were collected from the participants during the 4-week study period. Two samples were first taken at 1-week intervals to determine the base-line bacterial levels (sample I, day 1, and sample II, day 7). On day 14 the subjects rinsed for 1 min with 10 ml of 0.2% chlorhexidine-gluconate mouthwash (Hibitane Dental®, ICI, Macclesfield, England) 45 min before the sampling (sample III). The next sample was taken on the day after rinsing (sample IV, day 15). Two additional samples were taken 1 week (sample V, day 21) and 2 weeks after chlorhexidine-rinsing (sample VI, day 28).

The samples

The method for measuring the magnitude of microflora on oral epithelial surfaces has been presented previously (2, 10–12). All the samples were collected at about 0900 h. An area of the buccal mucosa corresponding to about from the upper second premolar to the upper second molar was scraped gently with a dry cotton swab. Each swab was then placed in a vial containing 1 ml of physiologic saline and kept cooled on ice. Sample III (day 14) and sample IV (day 15) were taken from contralateral sides in sequence. The subjects were then asked to chew a piece of paraffin (Orion Diagnostica, Espoo, Finland) for 5 min and to expectorate the accumulated whole saliva into clean cups with a milliliter scale for measuring the volume. The saliva samples were cooled on ice and taken immediately to the laboratory for further processing.

Bacterial culture

Serial dilutions of saliva were made to $1:10^6$ and $1:10^5$ in phosphate-buffered saline (PBS) with pH adjusted at 7.5. A volume (0.1 ml) of each dilution was then dispersed on blood agar plates for aerobic culture for 24 h at +37 °C. The number of colonies identifiable with the naked eye was counted, using an illuminated background table, and expressed as colony-forming units per milliliter of saliva (CFU/ml). The numbers of alpha-hemolytic and non-hemolytic bacterial colonies were counted separately.

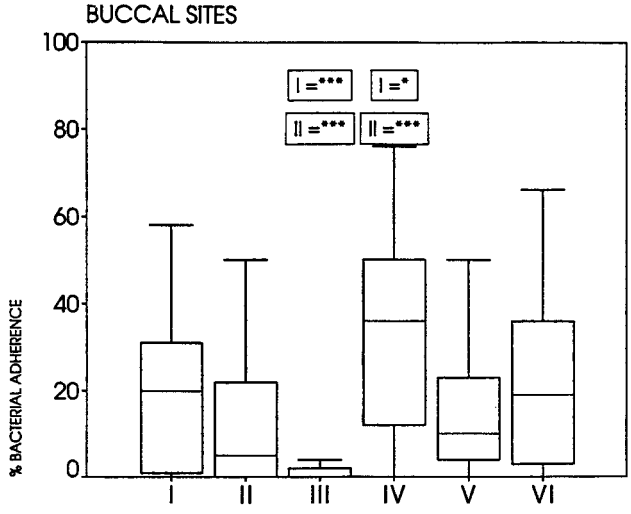

Fig. 1. Changes in bacterial adherence at buccal surfaces during the course of the study. The boxes are drawn to represent the interquartile range, and within them the medians are marked with lines. The whiskers extend as far as the most extreme values within 1.5 times the interquartile range. The statistical significance (one-sided) of contrast of variables by general linear model analysis of variance is indicated for each box plot as follows: $p < 0.005$ = ***; $p < 0.01$ = **; $p < 0.05$ = *. The upper value denotes the contrast against base-line sample I, and the lower the contrast against base-line sample II. Samples III and IV differ significantly from base-line samples.

Microscopy

The vials with the cotton swabs were vortexed at the maximum settings for 20 sec. Thereafter, the cotton swabs were rolled over microscopy glass slides to make smear preparations. Each microscopy slide was given a code for examiner blinding. The slides were air-dried at +37 °C, fixed with 95% ethanol, air-dried, and stored refrigerated at −70 °C. After being thawed and air-dried, the smear preparations were stained with acridine orange as described previously (9) and screened for epithelial cells under a fluorescence microscope (Leitz Labrolux, mounted with an Orthoplan objective and standard fluorescence equipment) at 500× magnification. For each slide 50 epithelial cells were counted and were dichotomized into subgroups of >50 bacteria/cell and cells with 0–50 bacteria. The percentages of epithelial cells with >50 adherent bacteria were used to obtain a figure for the magnitude of adherent bacteria (2, 10–12). The non-coccoid bacterial morphotypes, if present, were counted separately for each cell.

Identification of the cell-adherent bacteria

Additional epithelial cell samples were obtained from five healthy adult donors. The bacteria not attached to the buccal epithelial cells were separated from the epithelial cell suspension by filtrating the samples twice through a 5-μm filter (Sartorius, Minisart, Göttingen, Germany). The washed epithelial cells that were ar-

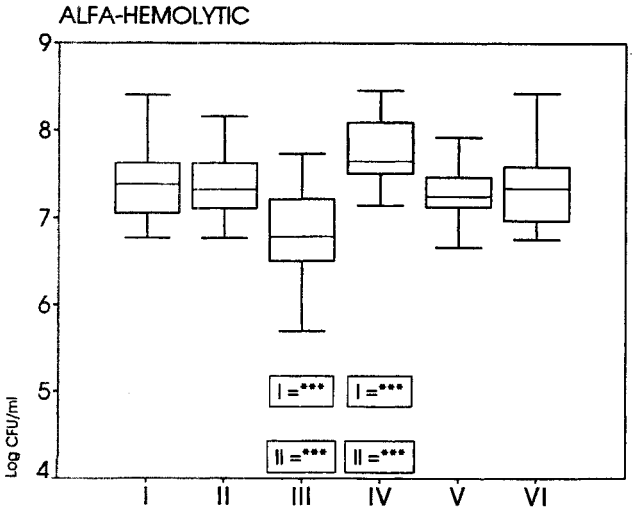

Fig. 2. The salivary alpha-hemolytic bacterial counts in the course of the study showed contrasts for samples III and IV. For details, see legend to Fig. 1.

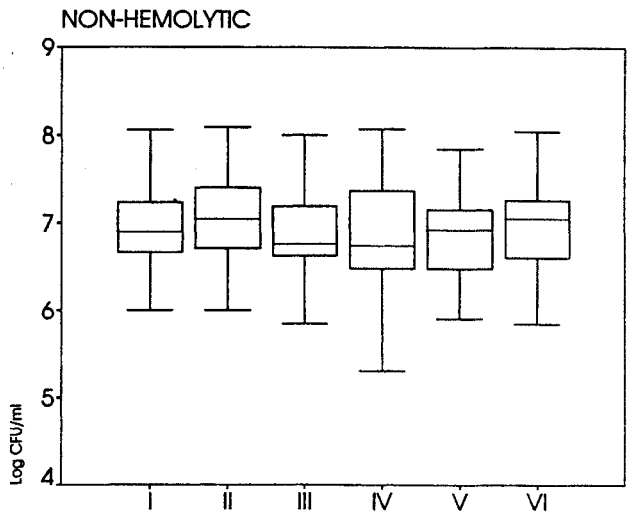

Fig. 3. The salivary non-hemolytic bacterial counts in the course of the study. For details, see legend to Fig. 1.

rested in the filter were suspended in PBS and cultured aerobically and anaerobically on blood agar plates. The resulting colonies were identified by colony morphology, hemolysis characteristics, oxidase reaction, and microscopy.

Analysis of data

A general linear model (GLM procedure, SAS Institute Inc.) was used to analyze the within-subject changes in repeated measurements. Base-line samples I and II were used as a control level with which the other samples were compared.

Spearman rank correlation tests were used to explore the effects of saliva flow rate on the mucosal and salivary bacterial levels. The relationships between the mucosal bacterial levels and the salivary CFU/ml counts were also examined with Spearman rank correlation tests.

Results

Mostly, the epithelial cell-adherent bacteria were slightly elongated coccoid morphotypes with very little size variation, assembled mainly in pairs, sometimes in short chains or microcolonies, thus resembling streptococci. In some rare cases low numbers of rod-formed organisms and occasional microcolonies of kidney-shaped bacteria of irregular size were also found. Aerobic and anaerobic culture of the additional, filtrated epithelial cell samples showed that practically all of the cell-adherent bacteria were streptococci of the viridans group, but about 1% of the colonies could be assigned to oropharyngeal *Neisseria* species (13). No strict anaerobes were present.

The data are shown as box plots in Figs. 1–3. After an initial decrease in the degrees of bacterial adherence after the chlorhexidine rinsing (sample III), an increase occurred on the day after the rinsing (sample IV). Salivary CFU counts of alpha-hemolytic streptococci showed a similar recoil-effect pattern, but no such change could be detected for the non-hemolytic salivary CFU counts.

The saliva flow rate of an individual was not significantly associated with the epithelial cell-adherent bacterial counts or with the salivary bacterial counts.

Discussion

The findings of this study are in agreement with our previous findings that only the streptococci-like organisms seem to be able to adhere to the epithelial cells of the oral mucosa, whereas rod-shaped and other morphotypes seem to be floating free. The culture of the filtrated epithelial cell samples confirmed that an overwhelming majority of the cell-adherent bacteria on oral mucosal surfaces were viridans streptococci. Of the many bacterial species that normally can be cultured from human saliva, only a fraction seems to be able to adhere to and colonize the oral epithelial surfaces (14). Recent studies suggest that *S. mitis* and *S. sanguis* are the most prevalent species dwelling on the human buccal oral mucosa (3, 6).

Chlorhexidine has a broad antibacterial spectrum, yet the sensitivities of oral bacterial strains differ widely. Although some oral viridans streptococci are suppressed by low (< 10 μg/ml, in vitro) concentrations of chlorhexidine, some strains—*S. sanguis* and *S. mitis*, for example—are much more resistant (15). Bonesvoll et al.

(16) showed that, whereas most of the oral chlorhexidine was eliminated in about 12 h, low, residual salivary concentrations persisted for 24 h after a single rinse with 0.2% chlorhexidine solution. It could be speculated that 1 day after rinsing, the concentrations of residual chlorhexidine would be at a critical level to suppress other naturally competing species except for these mucosa-adherent streptococci. Indeed, there are reports that suggest that *S. sanguis* could benefit from continuous chlorhexidine administration (17).

Previously, we reported an increase in mucosa-adherent streptococci lasting for up to 1 week (2). That long an effect could not be demonstrated in the present study, possibly because of the time span between the base-line sample (sample II) and the 1-week post-rinsing sample (sample V), which actually was 2 weeks in the present study, as compared with 1 week in the previous work (2). Nevertheless, it would be fair to assume that chlorhexidine becomes bound to oral mucosal epithelial cells in concentrations somewhere below the minimum inhibitory concentrations for most of mucosa-adherent viridans streptococci.

Normally, as chlorhexidine is usually administered twice daily, the concentration of chlorhexidine in the oral cavity remains high enough to suppress the total flora. However, the present study would indicate that some time after ceasing to use chlorhexidine mouthwashes an increase of commensal alpha-hemolytic streptococci may take place. Normally, the oral alpha-hemolytic flora can be considered beneficial for the patient in competitively preventing the establishment of pathogenic bacteria on mucosal surfaces (4, 5). However, increased amounts of indigenous bacteria adhering to host body surfaces may warrant careful consideration in oral hygiene protocols for patients at risk for nosocomial infections. The entrance of oral viridans streptococci into the blood circulation system via, for example, tooth extraction wounds is associated with the pathogenesis of bacterial endocarditis (9). Oral mucositis wounds in patients undergoing cytotoxic chemotherapy (7, 8) could be another port of entry of viridans streptococci into the circulation. Besides, the proliferation of dense layers of streptococci on the mucosal surfaces could be a feasible substrata onto which other, more pathogenic species may adhere (18, 19).

In conclusion, this study confirms our previous finding that a single rinse with chlorhexidine causes a delayed increase of oral epithelial cell-adherent bacteria. This increase seems to be strongly associated with the alpha-hemolytic portion of oral streptococci. Whether this microecologic recoil phenomenon is a risk or a benefit for the patient should be thoroughly examined.

References

1. Gjermo P. Chlorhexidine and related compounds. J Dent Res 1989;68:1602–8.
2. Vaahtoniemi LH, Räisänen S, Stenfors L-E. Effect of chlorhexidine and toothbrushing on the presence of bacteria on gingival and buccal epithelial cells. Oral Microbiol Immunol 1994;9:315–7.
3. Frandsen E, Predrazzoli V, Kilian M. Ecology of viridans streptococci in the oral cavity and the oropharynx. Oral Microbiol Immunol 1991;6:129–33.
4. Grahn E, Holm S. Penicillin concentration in saliva and its influence on bacterial interference. Scand J Infect Dis 1987;19: 235–41.
5. Roos K, Holm SE, Grahn E, Lind L. Alpha-streptococci as supplementary treatment of recurrent streptococcal tonsillitis: a randomized placebo-controlled study. Scand J Infect Dis 1993; 25:31–5.
6. Smith DJ, Anderson JM, King WF, van Houte J, Taubman MA. Oral streptococcal colonization of infants. Oral Microbiol Immunol 1993;8:1–4.
7. Arning M, Gehrt A, Aul C, Runde V, Hadding U, Schneider W. Septicema due to Streptococcus mitis in neutropenic patients with acute leukemia. Blut 1990;61:364–8.
8. Tasaka T, Nagai M, Sasaki K, Murata M, Taoka T, Ikeda K, et al. Streptococcus mitis septicemia in leukemia patients; clinical features and outcome. Intern Med 1993;32:221–4.
9. Nord CE, Heimdahl A. Cardiovascular infections: bacterial endocarditis of oral origin. Pathogenesis and prophylaxis. J Clin Periodontol 1990;17:494–6.
10. Stenfors L-E, Räisänen S. Age-dependent changes in bacterial adherence to epithelial cells of nasopharynx in vivo. Acta Otolaryngol (Stockh) 1990;110:292–9.
11. Stenfors L-E, Räisänen S. Abundant attachment of bacteria to nasopharyngeal epithelium in otitis-prone children. J Infect Dis 1992;165:1148–50.
12. Vaahtoniemi LH, Räisänen S, Stenfors L-E. The age-dependence of bacterial presence on oral epithelial surfaces in vivo. Oral Microbiol Immunol 1992;7:263–6.
13. Morello JA, Bohnhoff M. *Neisseria* and *Branhamella*. In: Lennette E, Balows A, Hausler WJ, Truant JP, editors. Manual of clinical microbiology, 3rd ed. Washington (DC): American Society for Microbiology, 1980:111–30.
14. Gibbons R, Van Houte J. Bacterial adherence in oral microbial ecology. Ann Rev Microbiol 1975;29:19–44.
15. Emilson CG. Susceptibility of various microorganisms to chlorhexidine. Scand J Dent Res 1977;85:255–65.
16. Bonesvoll P, Lökken P, Rölla G, Paus PN. Retention of chlorhexidine in the oral cavity after mouth rinses. Arch Oral Biol 1974; 19:209–12.
17. Emilson CG, Fornell J. Effect of toothbrushing with chlorhexidine gel on salivary microflora, oral hygiene, and caries. Scand J Dent Res 1976;84:308–19.
18. Skopek R, Liljemark W, Bloomquist C, Rudney J. Dental plaque development on defined streptococcal surfaces. Oral Microbiol Immunol 1993;8:16–23.
19. Kolenbrander P, London J. Adhere today, here tomorrow: oral bacterial adherence. J Bacteriol 1993;175:3247–52.

Received for publication 8 August 1994
Accepted 8 November 1994