

Feasibility of eradication of mutans streptococci from oral cavities

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Abstract: Objectives: Dental caries prevention programs using chlorhexidine (CHX) have been proposed, but CHX's effect in reducing levels of mutans streptococci (*S. mutans* and *S. sobrinus*) appears to last for only a few months. The aim of this study was to attempt to eradicate mutans streptococci from the oral cavity using intensive professional mechanical tooth cleaning (PMTC) and topical application of CHX in custom-made trays. **Methods:** Seven adult dentate subjects participated in this study (mean age 53.7 +/- 5.6, age range 46 to 62, mean DMFT, 9.1 +/- 4.2). For each subject, PMTC was carried out eight times within ten days. After each PMTC, 1% CHX was applied twice to the tooth surface using custom-made trays. In addition, as home treatment, subjects were required to carry out tooth brushing three times a day, and apply 0.2% CHX in custom trays after brushing in the morning and evening. In addition, subjects rinsed with 0.2% CHX solution after lunch. Salivary levels of mutans streptococci were evaluated using Dentocult-

SM at baseline and on days 9, 20, 70, 120. **Results:** Mutans streptococci were eradicated by day 120 from 4 of the 7 seven subjects participating in this study. Those 3 subjects still harboring mutans streptococci exhibited deep periodontal pocketing. **Conclusions:** Eradication of mutans streptococci from the oral cavity is feasible using a combination of CHX application in custom-made trays and intensive PMTC. (J. Oral Sci. 46, 179-183, 2004)

Keywords: mutans streptococci; chlorhexidine; eradication.

Introduction

It is generally accepted that harboring mutans streptococci (*Streptococcus mutans* and *Streptococcus sobrinus*) is a risk factor for dental caries. A number of in-vitro studies have demonstrated mechanisms by which these bacteria play a role in dental caries formation (1). In this regard, the production of water-insoluble glucans on the tooth surface seems a particularly important virulence factor (2-4).

Dental plaque is a biofilm, and the surface of it is covered with the matrix structure of dextran (5-8), which is resistant to penetration by most anti-microbial agents.

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Chlorhexidine (CHX) is a topical anti-microbial agent that is effective in the elimination of mutans streptococci from the oral cavity (9). Application of CHX is performed using a rinsing solution, dentifrice, varnish on the tooth surface or gel application in individual trays. Of these methods, varnish application to the tooth surface and gel application using individual trays have been shown to be most effective (10,11) since they permit a high concentration of CHX to be maintained on the tooth surface. However, even using these methods, this agent is still unable to penetrate the biofilm (11). Eradication of mutans streptococci using CHX has been attempted in the clinical trial setting, but re-growth of the bacteria is generally observed within two to three months (12). There are no reports of permanent eradication of mutans streptococci from the oral cavity. Moreover, attempting to eradicate these bacteria using high concentrations of CHX over an extended time frame may result in local adverse effects on the oral mucosa (13,14).

We propose use of a combination of professional mechanical tooth cleaning (PMTc) and a dental drug delivery system (3DS) with CHX for elimination of mutans streptococci from the oral cavity (15,16). 3DS consists of drug retainers that contact the tooth surface and permit contact of the anti-microbial drugs directly with the tooth surface. Since mutans streptococci do not have receptors to permit adherence to the oral mucosa (17), it is not necessary to eradicate them from areas where teeth are absent. It was hypothesized PMTC would destroy the biofilm structure and permit the delivered CHX to reach the mutans streptococci remaining in micro-colonies.

Materials and Methods

Subjects and clinical examination

This study was approved by the ethical committee of the National Institute of Infectious Diseases of Japan. Members of the institute and co-researchers of this study were recruited, and all subjects gave written informed consent for participation in the study. In total, seven adult male subjects participated, with mean age 53.7 ± 5.6 years and mean DMFT 9.1 ± 4.2 . Probing depths were measured at six sites per tooth using a WHO probe. Among the seven subjects, 2 subjects had deep periodontal pockets (> 4 mm). All subjects exhibited pockets of 3 mm.

Clinical and sampling procedures

Levels of salivary mutans streptococci were determined at baseline using Dentocult-SM strip methods (Orion Diagnostica, Finland). Alginate impressions were then taken and maxillary and mandible casts prepared. A polypropylene sheet (3.0 mm disk for mouth guard soft,

Keystone, New Jersey, U.S.A.) was vacuum-adapted to each cast with a vacuum-forming machine (VACCUM ADAPTER I, Keystone). Vacuum-adapted drug retainers were individually fabricated to cover the complete arch of the dentition. The drug retainer was trimmed to be approximately 1.0 mm apical to the gingival margin.

Before CHX application, PMTC was carried out eight times within 10 days on each subject to remove the tooth biofilm. By the PMTC, dental plaque was disclosed prior to its removal using rubber cups with polishing paste (Prophy Paste;(RDA170); CCS Cleanchemical, Vasby, Sweden). The remaining inter-dental plaque was removed using dental floss and polishing using Eva chips with polishing paste. Complete plaque removal was confirmed by further disclosing. The tooth surface was varnished with a 0.2% NaF solution (FULORIDENT GEL, Stone Pharmaceuticals, Philadelphia, USA).

After each PMTC, 1% CHX gel (CORSODYL Zahn-gel, Smithkline Beecham, Thorshaus, Switzerland) was injected into those periodontal pockets deeper than 4 mm. This gel was also applied using a dental drug retainer for 5 minutes. During the 10 days after PMTC, subjects also applied 0.2% CHX gel (Plakout, Howe-Neos Dental, Bioggio, Switzerland) twice a day after tooth brushing (morning and evening) using a custom-made tray. In addition, after lunch, tooth brushing and mouth rinsing using 0.2% CHX mouth rinse was performed. Salivary mutans streptococci levels were determined using the Dentocult-SM system at days 9, 20, 70, 120. On days 70 and 120, bacteria were also cultured to determine salivary mutans streptococci levels.

Microbial procedures and saliva sampling

Salivary mutans streptococci and Lactobacillus were counted using a commercially available mutans streptococci evaluation kit, Dentocult-SM (Orion Diagnostica, Epsom, Finland). The levels were classified according to the manufacturer's instructions, that is: level 0 - 1: $< 100,000$ colony forming units (CFU) mutans streptococci /ml saliva; level 2: $100,000 < \text{CFU/ml} < 1,000,000$; and level 3: $> 1,000,000$ CFU/ml.

On days 70 and 120, mutans streptococci were also cultured. Paraffin-stimulated whole saliva samples were collected for 5 minutes. Saliva samples of 50 μl were then sonicated by ultrasonic dispersion (60 power output) for 10 seconds and spread onto Mitis-Salivarius agar (MS, Gibco, Tokyo, Japan) plates for growth of streptococci, and onto improved Mitis-Salivarius agar plates containing 0.02 M bacitracin (Wako Pure Chemicals, Osaka, Japan) (MSB) for selective growth of mutans streptococci (18), using an EDDY JET spiral system (Gunze Sangyo, Tokyo,

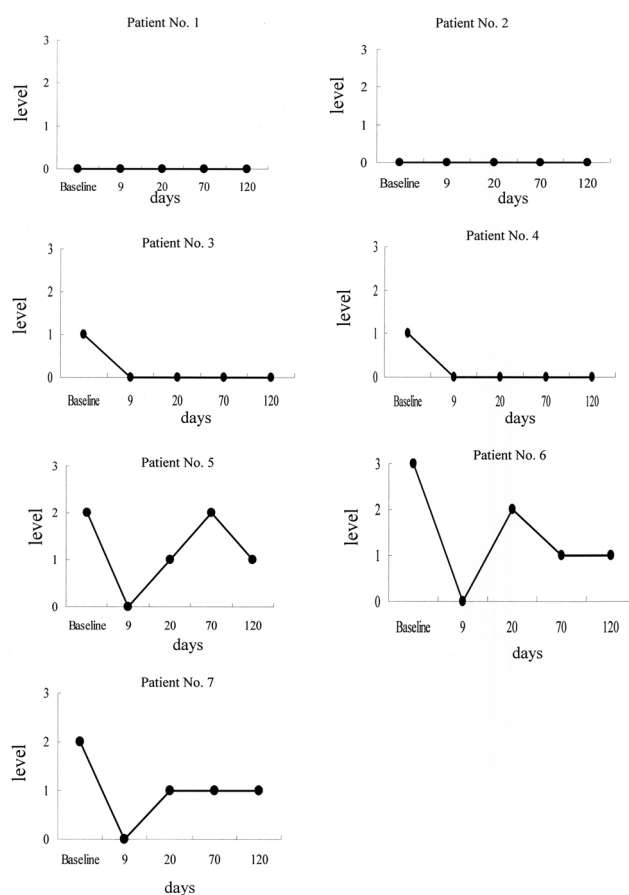


Fig. 1 Evaluation of the salivary levels of mutans streptococci by Dentocult SM.

Japan). After 48 hours anaerobic incubation, colonies were counted and the number of bacteria per ml of whole saliva calculated.

Statistical analysis

Mann-Whitney's *U*-tests were used for the evaluation of baseline differences between subjects in whom mutans streptococci was eradicated and those still harboring mutans streptococci.

Results

Figure 1 shows the mutans streptococci levels evaluated using the Dentocult SM. Two subjects with level 0 (no mutans streptococci detected) at baseline also had level 0 throughout the study. In addition, two subjects with level 1 at baseline decreased to the 0 level after 120 days. Three subjects above level 2 at baseline decreased to level 0 by day 9, and recovered to level 2 by day 120.

Figure 2 shows the mutans streptococci levels evaluated by the improved MSB culture system at 70 (A) and 120 days (B). Eradication of mutans streptococci was observed

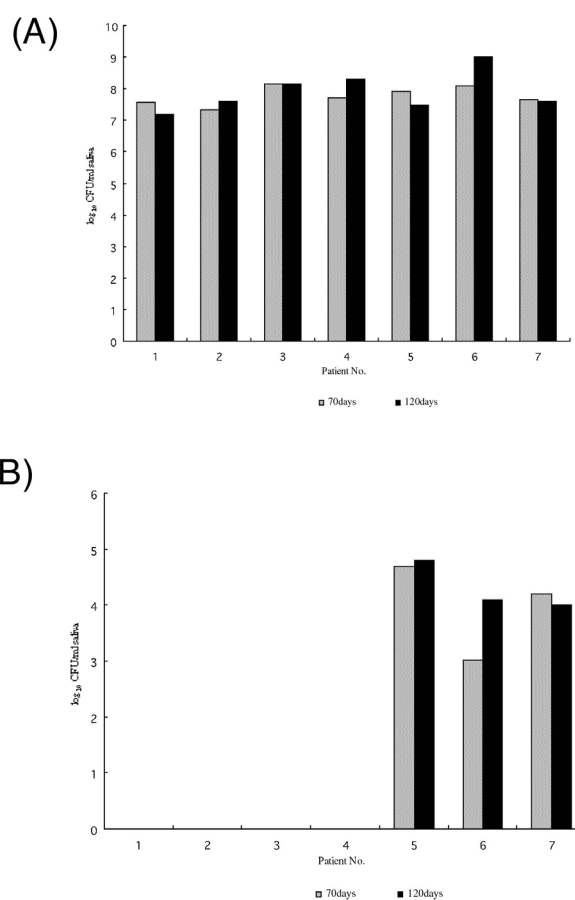


Fig. 2 Confirmation of the eradication of mutans streptococci from the oral cavity by culture.

Table 1 Baseline clinical characteristics (mean \pm SD) of subjects according to success of mutans streptococci eradication methods

	Eradicated (n = 4)	Not eradicated (n = 3)	P-value
DMFT	10.00 \pm 4.97	8.00 \pm 5.29	0.31
Probing depth (% of pockets)			
> 3 mm	49.85 \pm 19.92	54.76 \pm 45.07	0.78
> 4 mm	6.40 \pm 2.40	18.85 \pm 18.46	0.70
> 5 mm	1.64 \pm 0.89	6.94 \pm 9.57	0.71
> 6 mm	0.89 \pm 1.03	2.18 \pm 3.78	0.58

in three subjects.

The baseline characteristics of subjects in whom mutans streptococci were eradicated and those exhibiting mutans streptococci re-growth are shown in Table 1. There was a

tendency for those subjects with deep periodontal pockets to exhibit streptococci regrowth, although no statistically significant difference was observed.

Discussion

The results of the present study demonstrate that 3DS used in combination with intensive PMTC is effective in reducing salivary levels of mutans streptococci and in eradicating mutans streptococci. A number of dental caries preventive programs have been described (for review see Lewis et al.;19), including tooth cleaning, and fluoride or CHX application. Tooth cleaning and tooth brushing instruction have some benefit in caries prevention, although this benefit appears minor (20). A combination of oral hygiene instruction and fluoride application appears more effective (21), and if CHX is used in addition, an even greater favorable effect is evident (11). However, these preventive measures do not appear to result in complete inhibition of new dental caries.

Complete removal of dental plaque can be challenging, even in the case of chemical plaque removal (22). Tooth brushing in combination with tooth brushing instruction is also of questionable value, especially on tooth surfaces prone to caries (23). PMTC has been suggested as being one of the most effective methods for plaque removal, and PMTC performed by dental hygienists has been demonstrated to suppress dental caries (24,25) and reduce salivary levels of the mutans streptococci without the use of anti-microbial agents (26).

Elimination of mutans streptococci using CHX has been attempted in a number of studies, but the mean levels of these bacteria returned to baseline levels within two weeks when a rinsing solution was used (27), within 4 weeks using gel application or within 12 weeks using varnish (28). The results of these studies suggest that eradication of mutans streptococci from the oral cavity is not feasible. However, these results might be a consequence of inadequate plaque removal prior to CHX application. The dental plaque biofilm in which mutans streptococci are found (8) is resistant to anti-microbial agent penetration (29). Using the system described in the current study, pre-treatment of the tooth surface using PMTC to remove the biofilm appears to permit CHX to function more effectively in mutans streptococci removal.

The use of either PMTC or anti-microbial agents alone has some benefit in preventing dental caries, but is not effective in eradication of mutans streptococci. Using the system described in this study, that is complete removal of dental plaque by intensive PMTC and frequent applications of CHX within a short period of time, in some cases results in the eradication of mutans streptococci

from the oral cavity. However, even using this system, mutans streptococci were eradicated from only 4 of 7 subjects.

Baseline differences in the periodontal condition between subjects in whom mutans streptococci were eradicated and those in whom they were not eradicated were not seen. This may be the result of the small sample size, and thus insufficient power to detect differences. However, in general, those subjects where mutans streptococci were not eradicated had deeper periodontal pockets. These results suggest periodontal treatment might be advisable prior to use of anti-microbial agents.

In conclusion, using appropriate methods, eradication of mutans streptococci from the oral cavity is feasible, but is not consistently achieved.

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