Journal of Oral Science, Vol. 48, No. 3, 111-116, 2006

Original

# Study of the clinical usefulness of a dental drug system for selective reduction of mutans streptococci using a case series

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(Received 10 May and accepted 4 July 2006)

**Abstract: The aim of this study was to evaluate the clinical efficacy and safety of a dental drug delivery system (3DS) for the selective reduction of mutans streptococci. Twenty patients with high levels of mutans streptococci in saliva participated. The efficacy of 0.2% chlorhexidine (CHX) delivered by 3DS in reducing the salivary levels of mutans streptococci compared with total streptococci and lactobacilli was investigated. Each subject was treated by professional mechanical tooth cleaning (PMTC) and subsequently individual trays with CHX for 5 min. Salivary bacterial samples were taken at the baseline and weeks 1-12. A significant reduction in the colony count of mutans streptococci was observed during the first 4 weeks compared with the baseline count, while no significant decrease in the count of total streptococci or lactobacilli was found during 12 weeks. In particular, the proportion of mutans streptococci in total streptococci remained low after 12 weeks. Our results indicate that the new 3DS used in combination with PMTC appears to be a promising intraoral drug delivery system which, when used with a low CHX concentration selectively, effectively reduces mutans streptococci in the oral cavity with no adverse effects. (J. Oral Sci. 48, 111-116, 2006)**

Keywords: chlorhexidine; dental drug delivery system; drug retainer; mutans streptococci; professional mechanical tooth cleaning; saliva.

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## Introduction

Harboring mutans streptococci (*Streptococcus mutans* and *Streptococcus sobrinus*) has been recognized as a risk factor for dental caries, and reduction of these bacteria is a preventive strategy for this disease. Chlorhexidine (CHX) is an effective topical antimicrobial agent for eliminating mutans streptococci from the oral cavity (1). Although elimination of mutans streptococci using CHX has been attempted in a number of studies, the mean levels of these bacteria returned to the baseline levels within two weeks when a rinsing solution was used (2), within 4 weeks when applied as a gel, or within 12 weeks using a varnish (3). These results may be due to the fact that the dental plaque biofilm in which mutans streptococci inhabit resists penetration of antimicrobial agents (4,5). On the other hand, attempts to eradicate these bacteria using high concentrations of CHX over an extended time frame may result in local adverse effects on the oral mucosa (6,7).

Therefore our group has proposed that a combination of professional mechanical tooth cleaning (PMTC) and CHX delivered by a dental drug delivery system (3DS) may be effective for elimination of mutans streptococci from the oral cavity (8-10). We hypothesize that PMTC destroys the biofilm structure and permits the delivered CHX to reach and act on micro-colonies of mutans streptococci. The 3DS consists of drug retainers covering the tooth surface and permits direct contact of antimicrobial drugs with the tooth surface. Since mutans streptococci do not have receptors for adhesion to the oral mucosa (11), there is no need to treat areas where teeth are absent. The present study attempted to eradicate mutans streptococci from the oral cavity using intensive PMTC and topical application of CHX in custom-made trays, and evaluated the clinical efficacy and safety of CHX delivered by the 3DS.

## Materials and Methods

### Subjects

This study was approved by the ethical committee of the National Institute of Infectious Diseases of Japan. All patients attending a private dental clinic gave written informed consent to participate. At screening, salivary mutans streptococci were counted using a commercially available mutans streptococci evaluation kit, Dentocult-SM (Orion Diagnostica, Epsom, Finland). The levels of mutans streptococci were classified according to the manufacturer's instructions as follows: level 0: $< 10^4$ colony forming units (CFU)/ml saliva, level 1: $< 10^4$–$10^5$ colony forming units CFU/ml saliva; level 2: $10^5$–$10^6$ CFU/ml saliva; and level 3: $> 10^6$ CFU/ml saliva. Patients classified as level 2 or above were recruited for the study. Two patients who were smokers and one pregnant woman were included. A total of five adult males and fifteen females participated, with a mean age of 39.9 ± 14.0 years (range; 22 to 66 years). None of the subjects had missing teeth, except for the third molars. The subjects had not taken antibiotics for at least six weeks before saliva sampling or during the experimental period. All subjects used a fluoride dentifrice twice a day.

### Dental drug delivery tray fabrication

At the time of screening, alginate impressions were taken from the patients, and maxillary and mandibular casts were prepared. Each tooth on the cast was blocked with paraffin wax to obtain space for drug delivery. A polypropylene sheet (3.0 mm disk for soft mouth guard, Keystone, New Jersey, USA) was vacuum-adapted to each cast with a vacuum-forming machine (Vacuum Adapter I, Keystone). From the vacuum-adapted sheets, individual trays, referred to as drug retainers, were made to fit onto the tooth surfaces to cover the complete arch of the dentition (8-10). The drug retainer was trimmed to be approximately 1.0 mm above the gingival margin.

### Clinical procedures

At the first visit, a paraffin-stimulated 5-min whole saliva sample was obtained for determination of baseline data, and the volume of the saliva sample was measured.

At the next visit, the patient was given CHX treatment delivered by 3DS. Before 3DS application, PMTC was performed to remove the biofilm on the tooth surfaces. Supragingival calculi were removed using an ultrasonic scaler (Supuason Satelec Inc., Bordeaux, France). The tooth surfaces were subsequently treated with a disclosing agent, and dental plaques were removed by brushing and flossing. These procedures were performed several times until the tooth surfaces were no longer colored with the disclosing agent. After the plaque had been removed completely, a fluoride-containing polishing paste (Melsurgu Fine or Regular; Shofu Inc., Tokyo, Japan) was applied using a rotating rubber cup (Prophy Cup, Eiko Co. Ltd., Tokyo, Japan) or a rotating brush (Mini brush; Hawe-Neos Dental, Bioggio, Switzerland). A 0.2% commercially available chlorhexidine gel (Plakout, Howe-Neos Dental, Bioggio, Switzerland) was then applied to the tooth surface using the dental drug retainer for 5 min. The retainer was then removed, and the gel remaining on the tooth surfaces and in the inter-dental spaces was removed by rinsing with water and hand flossing. The subject was advised not to eat or drink for 2 h after the treatment. After 3DS application, the subject was given a new toothbrush to prevent reinfection by mutans streptococci. In addition, as home care, the subject was advised to apply a commercially available 0.4% stannous fluoride gel (Homejel; Oral Care Co. Ltd., Tokyo, Japan) using the drug retainer for five min twice a day after brushing.

One week after the first treatment, 3DS was applied again at the clinic. Then, at 1, 2, 3, 4, 9 and 12 weeks after completion of the 3DS treatment, paraffin-stimulated whole saliva samples were collected for microbial culture. The salivary level of mutans streptococci was determined again using the Dentocult SM system at 12 weeks.

### Microbial procedures

At the baseline and at 1, 2, 3, 4, 9 and 12 weeks after 3DS application, paraffin-stimulated whole saliva samples were collected for 5 min. The obtained saliva samples were immediately brought to the laboratory, vortexed for 30 s, and diluted 1:$10^2$–$10^4$ in phosphate-buffered saline. Fifty-microliter aliquots were spread onto Mitis-Salivarius agar plates (MS; Difco, Tokyo, Japan) for culture of total

streptococci, improved Mitis-Salivarius agar plates containing 0.2 U bacitracin (Wako Pure Chemicals, Osaka, Japan) (MSB) for selective culture of mutans streptococci, and Rogosa SL agar plates (Nippon Becton Dickinson Co. Ltd., Tokyo, Japan) for selective culture of lactobacilli. Spreading was done using an Eddy Jet spiral system (Gunze Sangyo, Tokyo, Japan). After 48 h of anaerobic incubation, colonies were counted and the number of bacteria per ml of saliva calculated based on the colony-forming units (CFU).

Salivary mutans streptococci levels were determined using the Dentocult SM system at screening and again at 12 weeks after treatment.

### Adverse reactions

The subjects were examined at each visit for adverse reactions in the oral cavity. Tooth and mucosal staining was checked by comparison with the photographs taken at the baseline. The subjects were questioned prior to clinical examination about the occurrence of any adverse reactions, such as a burning sensation in the oral mucosa.

### Statistical analysis

The bacteriological counts were $\log_{10}$-transformed prior to statistical analysis to normalize the variances. All values were expressed as means ± SD. The treatment effect in each period compared with the baseline was evaluated with Wilcoxon signed-rank test. Differences at $P$ values of 0.05 or less were considered to be statistically significant.

## Results

The prevalence of dental caries in the study group (mean DMF ± SD) at the baseline was 17.20 ± 6.20. No adverse effects were observed during the experimental period.

The mean salivary total streptococci, mutans streptococci, and lactobacilli counts at the baseline and during follow-up are shown in Table 1. No significant changes in the count of total streptococci were found using the culture system except at weeks 3 and 12 compared to the baseline. At 3 and 12 weeks, only slight but significant increases were found. The lactobacilli count also increased slightly but significantly at 1, 2, 3, and 4 weeks. However, the counts returned to the baseline level at 9 and 12 weeks. No decrease in total streptococci and lactobacilli was observed during the study period. The counts of mutans streptococci were clearly reduced after a week compared to the baseline, and significant decreases continued for four weeks. Furthermore, the proportion of mutans streptococci in total streptococci was reduced immediately after treatment and remained low even after 12 weeks (Table 1). The differences were statistically significant ($P < 0.05$) compared to the baseline except at 2 and 12 weeks.

The data for individual subjects are presented in Fig. 1. When the level of mutans streptococci in saliva was determined by the Dentocult-SM strip method, 16 of the 20 subjects showed decreases in level at 12 weeks compared to the baseline, and the difference was statistically significant ($P < 0.001$) (Fig. 1A). The MSB culture results indicated that the salivary mutans streptococci counts were reduced in 11 subjects, remained unchanged in 5 and increased slightly in 4 (Fig. 1B). Furthermore, when the proportion of mutans streptococci in total streptococci was evaluated, a decrease compared to the baseline was observed in all but four of the subjects (Fig. 1C).

## Discussion

Several methods using CHX have been reported to eliminate mutans streptococci from the oral cavity. Mouth rinses (12-14), dentifrices (15,16) and varnishes (17-19) have all been found to reduce salivary mutans streptococci levels. The effect of mouth rinsing in reducing mutans streptococci lasted only one week (13) and the efficiency depended upon the concentration (14). Varnish was the most effective method of applying CHX to the dentition, and suppressed mutans streptococci for five weeks (18). However, it was necessary to use a high concentration of CHX (40%) to obtain a significant reduction compared with placebo. In the present study, we examined the clinical efficacy of 0.2% CHX delivered by a dental drug delivery system (drug retainer) in reducing mutans streptococci on tooth surfaces in adults. The drug retainer was an apparatus that has been used in previous experiments for bacterial elimination (20-24). Randomized control trials demonstrated that individual trays were more effective than other application methods such as polishing the tooth surfaces with gel containing CHX (24) or brushing with a dentifrice containing CHX (20). Using individual CHX-coated trays worn overnight by subjects during sleep for one week, Hildebrandt et al. (21) reported significant reduction of mutans streptococci in saliva lasting three months in adults (21) and four months in children (23). In another study, application of a 1% CHX gel by individual trays reduced mutans streptococci in saliva for only three days in children with high mutans streptococci levels in their saliva (24). Compared with these studies, our study achieved reduction of mutans streptococci counts in saliva for twelve weeks with significant reduction for four weeks. Achong et al. (23) obtained a significant reduction of mutans streptococci in saliva for four months; however, the CHX was applied simultaneously with caries restorative treatment.

Only one of these previous studies used mechanical

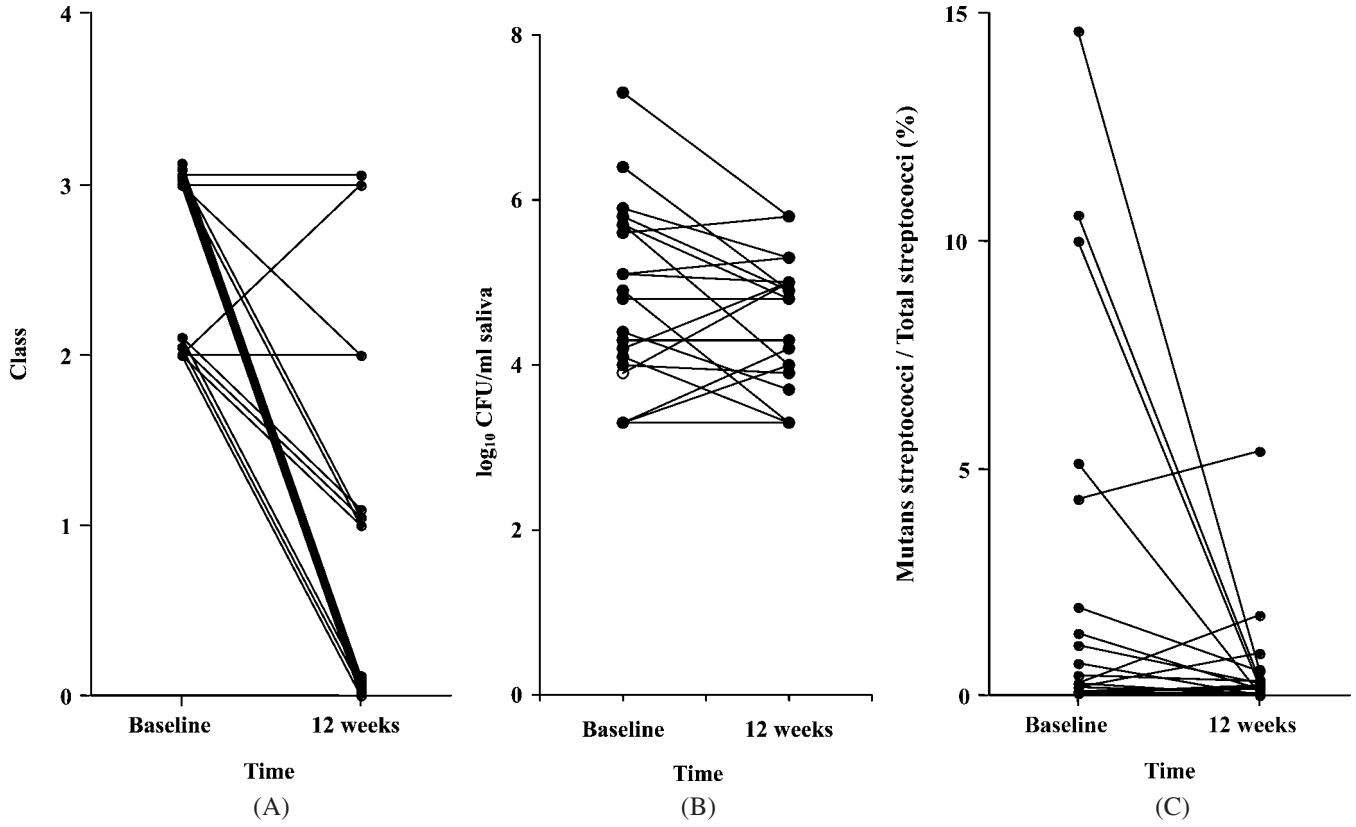


Fig. 1 Data of individual subjects (n = 20) on changes in salivary mutans streptococci levels determined by Dentocult SM (A), culture on improved MS agar (B) and proportion of mutans streptococci in total streptococci (C) before and after treatment using 3DS.

Table 1 Changes in colony counts (CFU) of mutans streptococci, lactobacilli, total streptococci and the proportion of mutans streptococci in total streptococci before and after the treatment using 3DS

| | Baseline | Time (weeks) after completing treatment using 3DS | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 9 | 12 |
| Mutans streptococci | 4.9 (1.1) | 3.7 (0.46)* | 4.2 (0.76)* | 4.3 (0.78)* | 4.3 (0.74)* | 4.4 (0.97) | 4.5 (0.80) |
| Lactobacilli | 4.2 (0.81) | 4.6 (1.10)* | 4.5 (1.10) * | 4.7 (1.20)* | 4.5 (0.87)* | 4.6 (1.39) | 4.4 (1.04) |
| Total streptococci | 7.1 (0.45) | 6.9 (0.43) | 7.1 (0.64) | 7.3 (0.51)* | 7.2 (0.43) | 7.3 (0.53) | 7.4 (0.32)* |
| Proportion of S.mutans (%) | 2.6 (4.26) | 0.1 (0.11)* | 0.4 (0.75) | 0.2 (0.24)* | 0.5 (1.01)* | 0.5 (1.24)* | 0.6 (1.20) |

Values are mean (SD) of $\log_{10}$ CFU/ml saliva (n = 20). CFU were determined by conventional culture method.

*: significant difference versus baseline ($P < 0.05$)

tooth cleaning as a pretreatment (24). The dental plaque biofilm that contains mutans streptococci (4) is resistant to penetration by antimicrobial agents (5). In addition, several studies have supported the view that recolonization of mutans streptococci on tooth surfaces disinfected with CHX is due to regrowth of organisms that are not eradicated, rather than the introduction of new organisms from an external source (25). For this reason, pretreatment of the tooth surface using PMTC to remove the biofilm is important to achieve more effective penetration of CHX. We suggest that combined use of PMTC and an antimicrobial agent provides an effective preventive system. This method permits the use of a relatively low concentration of CHX.

A number of previous studies have demonstrated that CHX does not affect the numbers of lactobacilli and total streptococci in the oral cavity (14,16). As in previous studies with CHX, the level of total streptococci was not

affected by the experimental treatment in this study. CHX delivered by the 3DS is fairly specific in suppressing mutans streptococci in the oral flora, because the 3DS is in direct contact with the tooth surface that primarily harbors mutans streptococci. Only minor adverse effects are associated with 3DS, since CHX does not come into contact with the oral mucosa. No traces of brown stain were found on the teeth of the subjects during the experimental period. In this study, no subject complained of a burning sensation in the oral mucosa or any change in the taste of food. Of course, no keratinization of the oral mucosa was observed, since CHX was applied for only twenty minutes and the concentration was lower than in previous studies. A single application of low-concentration CHX gel confers strong initial suppression, but the effect lasts for only a short period (18,26). Long-term suppression of mutans streptococci is achieved by repeated application of CHX. However, repeated applications, even using individual trays, are sometimes accompanied by adverse effects such as a burning sensation in the oral mucosa and a change in the taste of food (22). The absence of any side effects of the 3DS in this study may be explained by the minimum contact between CHX and the oral mucosa.

In conclusion, our results indicate that the 3DS used with PMTC appears to be a promising intraoral drug delivery system. The 3DS used with a low CHX concentration selectively and effectively reduces mutans streptococci in the oral cavity with no adverse effects.

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