Pilot study of the effect of a new dental drug delivery system for removal of mutans streptococci infection in adults

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Purpose: The aim of this study is to evaluate the clinical efficacy and safety of a new dental drug delivery system (3DS) for the selective reduction of mutans streptococci, a potent risk factor for dental caries.
Materials and Methods: Twelve patients were assigned to two groups, professional mechanical tooth cleaning (PMTC) with or without 3DS. The efficiency of 3DS with 0.2% chlorhexidine (CHX) in reducing the salivary levels of total Streptococci, mutans streptococci, and Lactobacilli, was investigated. The subjects in the 3DS combination group were treated with PMTC and subsequently individual trays with CHX for five minutes. Salivary bacterial samples were taken at baseline and in weeks 1, 2, 3, 4, and 8.
Results: Non-significant changes in the total Streptococci, mutans streptococci, and Lactobacilli counts were found during the effects of two groups in the study period ($p>0.05$). However, the proportion of mutans streptococci in total Streptococci at 3DS group was still low after 8 weeks compared with baseline, but not significant.
Conclusion: The results indicate that 3DS in combination with PMTC is an effective intraoral drug delivery system that specifically reduces mutans streptococci, especially in proportion of the total streptococci without any adverse effects. **(Asian Pac J Dent 2013; 13: 11-18.)**
Key Words: chlorhexidine, dental drug delivery system, drug retainer, mutans streptococci, PMTC, saliva

Introduction

The principal microorganism associated with the development of dental caries is mutans streptococci including *Streptococcus mutans* and *Streptococcus sobrinus*.[1,2] Mutans streptococci tenaciously colonize smooth surfaces rather than the oral mucosa.[3] Efficient prevention for the dental caries will be possible if mutans streptococci can be removed from the tooth surface. Some studies demonstrated clinical application of anti-microbial agents and application methods reduce the amount of mutans streptococci in the oral cavity.[4-8] Chlorhexidine (CHX) is an anti-microbial agent frequently used for chemotherapy against mutans streptococci on human dentition, and its ability to reduce caries in humans has been well documented.[5] Mutans streptococci can be reduced successfully for various periods of time with gels and novel solutions containing CHX, depending on the frequency of exposure and the concentration of CHX.[9] However, the effect of CHX on microorganisms is so strong that there is a risk of changing the oral microflora and microbial substitution must be carefully evaluated. Some clinical trials have shown that long time use of CHX can cause tooth staining or a burning sensation in the oral mucosa or on the tongue.[10,11] Stratum corneum and keratinization of the tongue have also been reported.[12]

Therefore, our group has proposed that a combination of professional mechanical tooth cleaning (PMTC) and CHX delivered by the dental drug delivery system (3DS) is an effective method for elimination of mutans streptococci from the oral cavity.[8,13,14] The dental plaque biofilm that contains mutans streptococci on the tooth surface is covered with glucans strongly and resists the penetration of anti-microbial drugs[15] with large molecular weight. The biofilm must be removed when using anti-microbial drugs, PMTC eliminates the biofilm

from the tooth surface.[16] We hypothesize that PMTC destroys the biofilm structure and permits the delivered CHX to disinfect the recolonization of mutans streptococci, and eradicated regrowth of organisms. The 3DS uses individual trays called drug retainers that apply the anti-microbial drugs onto the dentition immediately after PMTC. The drug retainer minimizes contact of the anti-microbial drugs to the oral mucosa[17] and optimizes its effect for the tooth surface without dilution by saliva. Our previous report on 3DS using 0.2% CHX indicated that mutans streptococci was controlled in adults by the series studies.[8,13]

However, we did not set up the control group; therefore previous reports were all case series study. Furthermore, it is a result of obtaining with a combination with PMTC though the effect of 3DS is admitted. PMTC is also effective for the reduction of mutans streptococci for prevention of dental caries. In this study, we designed two groups with or without the drug delivery. Thus, it is necessary to compare the effect of 3DS with the PMTC only for the evaluation of the true effect of 3DS, and evaluated the clinical efficacy of CHX delivered by the 3DS. Moreover, it is added to calculate effective sample size in further study about the difference of the effect of two groups.

Materials and Methods

Subjects

Study population consisted of twelve adult subjects, five males and seven females, who attended to a private dental office for the regular check up and tooth cleaning, mean age was 34.7+/-11.9 years (range: 22 to 58 years). No subjects who participated in this study had missing teeth in their dentition, except for the third molars. Twelve subjects gave written informed consent to participate, and they were screened for salivary levels of mutans streptococci by the chair-side strip method (Dentocult-SM Orion Diagnostica Co., Ltd., Epso, Finland). The levels were classified according to the manufacturer's instructions, that is level 0 - 1: <100,000 colonies forming units (CFU) mutans streptococci/mL saliva; level 2: 100,000-1,000,000 CFU/mL,; and level 3: >1,000,000 CFU/mL. They were selected based on their having salivary levels of mutans streptococci more than level 2, which indicated that caries activity is high. We randomly allocated them to two groups. One group was received 3DS after PMTC, and another groups were received only PMTC. All subjects had good oral hygiene and no caries lesions were clinically detectable. None of the subjects wore removable dentures. All who participated in this study used a fluoride dentifrice twice a day. No subject regularly took anti-microbial medication or prescription drugs before or during the experimental period. This study was approved by the ethical committee of the Tsurumi University of Dental Medicine (No. 409, November 30, 2006).

Fabrication of dental drug delivery tray

For the subjects in groups of the doing 3DS, their maxillary and mandibular casts were prepared from the alginate impression. Each tooth was blocked out with paraffin wax on the cast to obtain space for the drug delivery. A polypropylene sheet (3.0 mm disk for soft mouth guard, Keystone Co., Ltd., Cherry Hill, NJ, USA) was vacuum adapted to each cast with a vacuum-forming machine (Vacuum Adapter I, Keystone Co., Ltd.). Vacuum-adapted drug retainers were individually fabricated to cover the complete arch of the dentition. The drug retainer was trimmed approximately 1.0 mm apical to the gingival margin.

Clinical procedures

At first, five-minute paraffin-stimulated whole saliva samples were obtained at the first appointment and the volume of each saliva sample was measured.

For twelve, all subjects as the following, supragingival calculus was removed by an ultrasonic scaler (Suprasson, Satelec Inc., Bordeaux, France). PMTC was carried out before 3DS treatment to remove the dental plaque biofilm on the tooth surfaces. Initially, dental plaque was removed by brushing and flossing, performed several times until the tooth surfaces were no longer disclosed by Prospec Plaque Disclosing Solution (GC Corp., Tokyo, Japan). After the plaque had been removed completely, a fluoride-containing polishing paste (Melsurgu Fine or Regular, Shofu Inc., Kyoto, Japan) was applied by rotating rubber cups (Prophy Cup, Eiko Co., Ltd., Tokyo, Japan) or a rotating brush (Mini Brush, Hawe-Neos Dental, Bioggio, Switzerland). The medical treatment performed to PMTC only group was above.

Then, for the 3DS group subjects only, a 0.2% commercially available chlorhexidine gel (PlaK Out; Kerr Hawa SA, Bioggio, Switzerland) was applied by a drug retainer to the dentition for 5 minutes. The gel remaining on the tooth surfaces and in the inter-dental spaces was removed by rinsing with water and hand flossing. This process was then repeated. This protocol was carried out again within 1 week. The subjects were advised not to eat or drink for two hours after the treatment. After 3DS, to prevent reinjection by mutans streptococci that adheres to toothbrush daily used, all subjects were provided with a new toothbrush. As home care, the subjects in 3DS group were advised to apply a commercially available 0.4% stannous fluoride gel (Homegel, Oral Care Co., Ltd., Tokyo, Japan) by drug retainer for 5 minutes twice a day after brushing. Subsequent paraffin-stimulated whole saliva samples were taken at 1, 2, 3, 4, and 8 weeks after the treatment. Salivary levels of mutans streptococci were checked again at the 8 weeks appointment by microbial cultures.

Microbial procedures

At the first appointment as a baseline data, paraffin-stimulated whole saliva samples were collected for five minutes before series treatments. The obtained saliva samples were immediately brought to the laboratory and vortexed for 30 s and diluted $1/10^2$-10^4 in phosphate buffered saline. To quantify the salivary levels of total Streptococci, mutans streptococci and Lactobacilli in saliva, microbial procedures were carried out according to methods described previously.[18-20] Briefly, 50.0 μL of saliva samples were vortexed for 10 s and inoculated onto Mitis-Salivarius agar (MS: Difco, Tokyo, Japan) medium for total Streptococci counts. The modified MSB agar medium[18] contained MS agar (Difco, Tokyo, Japan) supplemented with 20% sucrose (Wako Pure Chemicals Co., Osaka, Japan), 20 mg/mL Yeast Extract (Becton Dickinson, Sparks, MD, USA), 0.25 U bacitracin (Sigma, St. Louis, MO, USA), 10 mg/mL colistin (Wako Pure Chemicals Co.), 10 mg/mL nalidixic acid (Wako Pure Chemicals Co.), 4 mg/mL gramicidin (Sigma), and 1% tellurite solution for mutans streptococci. Rogosa SL agar (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) medium[21] was used for Lactobacilli. Eddy Jet spiral system (Gunze Sangyo, Inc., Tokyo, Japan) was used for bacterial counting. After anaerobic incubation for 42 hours for total Streptococci and the mutans streptococci, and for 72 hours for Lactobacilli, the visible colonies grown on these media were counted using a spiral systems counting grid. Then colony count data were transformed to lg. CFU/mL in saliva. In addition to baseline data, the quantity of bacteria was measured similarly at 1, 2, 3, 4, and 8 weeks in the study period.

Experiences of adverse effect

The subjects were examined at each visit for adverse reactions in the oral cavity. Tooth and mucosal staining was checked by using photographs taken at the baseline. The subjects were questioned prior to clinical examination about the occurrence of any adverse experiences, 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 +/- standard deviation (SD). The treatment effect in each period compared with the baseline values was evaluated with Willcoxon-sighned rank test. Differences between PMTC only and 3DS combination groups were analyzed using the Mann-Whitney U test. P values of 0.05 or less were considered to indicate statistical significance. These analyses were carried using SPSS software Ver17.0 (SPSS Co., Ltd., Tokyo, Japan). Then we used the power analysis to calculate the sample size to obtain the statistically significant difference between the two groups for further study (α=0.05, β=0.2) (S-Plus 6 for Windows, Insightful Corp., Seattle, WA, USA).

Results

Experience of dental caries was as follows: number of filled teeth ranged from 0 to 23, missing teeth 0, decayed teeth 0 by oral examination. No adverse effects were observed in the experimental period. No traces of brown stains were found on the teeth of surfaces through the experimental period. No subject complained of a burning sensation in the oral mucosa or any change in the taste of food. No keratinization of the oral mucosa was observed.

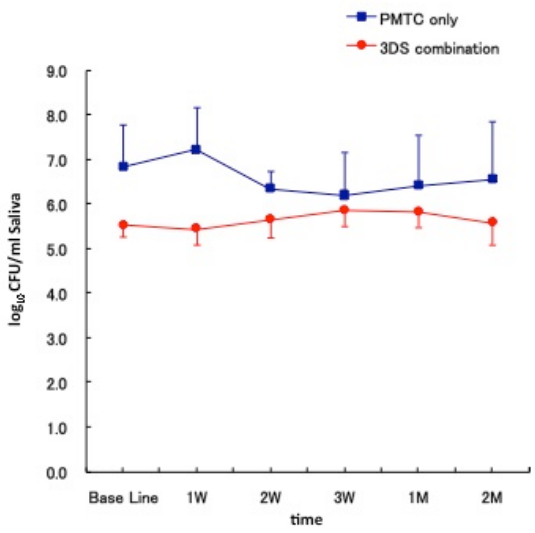

Fig. 1. Evaluation of total Streptococci for PMTC only and 3DS combination group, in the study period

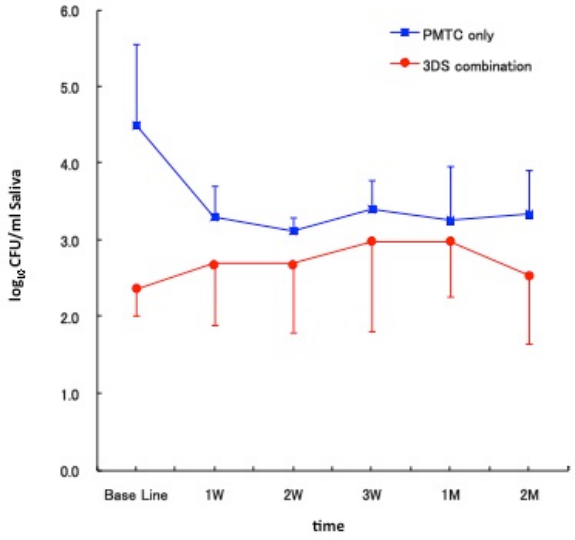

Fig. 2. Evaluation of the Lactobacilli for two groups, in the study period

The mean salivary level of total Streptococci, mutans streptococci and Lactobacilli counts at baseline and follow-ups are given in Figs. 1-3. At only PMTC group, the count of total Streptococci increased after one week, but two weeks later it decrease, and no significant changes were found by the culture system during the effects of two groups, the count of total Streptococci at the two months later returned toward baseline data (Fig. 1). Although the counts of Lactobacilli in only PMTC group were clearly reduced one week after the treatment, at 3DS combination group counts of the increased throughout the experimental period, but the changes in the counts were not statistically significant. There was a small decrease in the counts of Lactobacilli after two months (Fig. 2). Compared with the baseline values, the mutans streptococci counts in two groups were not statistically significant (Fig. 3). Figure 4 shows the course of the proportions of mutans streptococci in the total

Streptococci. In both groups, there were immediately and clearly reduced after one week the treatment. Then, in only PMTC group, it was gradually increased and amounted to more than baseline after two months. For the 3DS group, it was maintained at low level and control of proportion of mutans streptococci in the total Streptococci. However, the differences were not statistically significant during two groups through the experimented periods. And, it was clarified that it was necessary 30 subjects for each group, to obtain the statistically significant difference for 2 month between the two groups in further study.

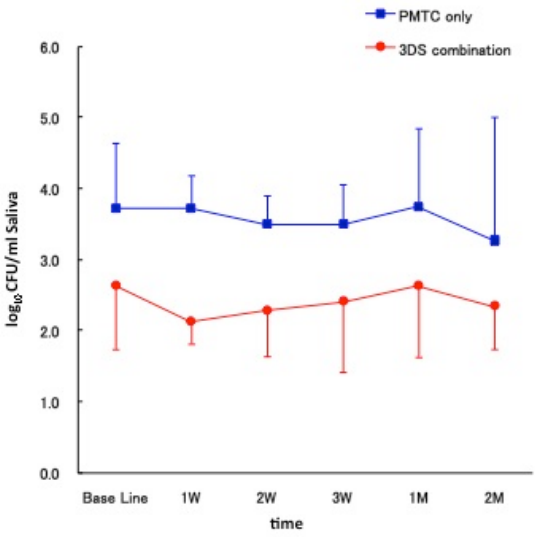

Fig. 3. Evaluation of the mutans streptococci for two groups, in the study period

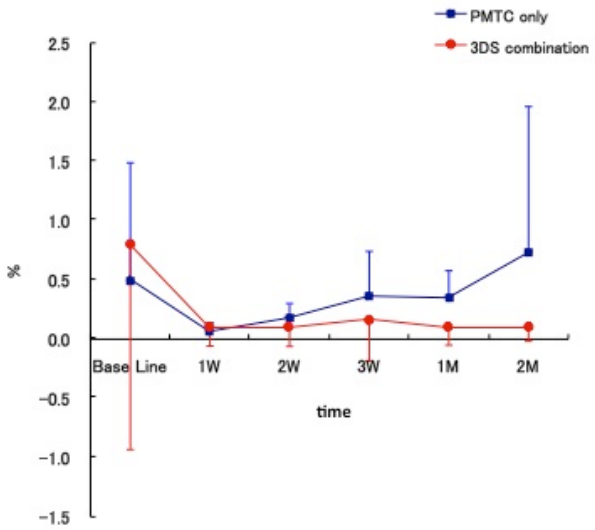

Fig. 4. Evaluation of the proportion of mutans streptococci in total Streptococci for each group, in the study period

Discussion

Some clinical methods using CHX were reported to eliminate mutans streptococci from the oral cavity. Mouth rinse,[18,19,21] dentifrice,[22,23] and varnishes[24-26] have all been found to reduce salivary levels of mutans streptococci. The effect of mouth rinse on the reduction of mutans streptococci lasted for only one week,[18] and the efficiency depended upon its concentration.[21] Varnish was the most effective method of applying CHX to the dentition and suppressed mutans streptococci for 5 weeks.[24] However, it was necessary to use a high concentration of CHX (40%) to obtain a significant reduction, compared with placebo varnish. The drug retainer was an apparatus that was used in previous experiments on bacterial elimination.[23,27-30] Some randomized control trials demonstrated that individual trays were more effective than other application methods such as polishing the tooth surfaces with a gel containing CHX[30] or brushing with a dentifrice containing CHX.[31] Hildebrandt et al.[28] reported that a significant reduction of mutans streptococci in saliva was achieved for 3 months in adults by using individual trays coated with CHX overnight for 1 week while the subjects slept and for 4 months in children.[29] Application of a gel containing 1% CHX by individual trays could reduce mutans streptococci in saliva for only 3 days in children with high mutans streptococci levels in their saliva.[30] We demonstrated previously the 3DS using 0.2% CHX used in combination with intensive PMTC is effective against mutans streptococci on tooth surfaces in adults.[32] Our study obtained mutans streptococci reduction in saliva for twelve weeks and the reduction was significant for four weeks. Only one of these previous studies used mechanical tooth cleaning as a pretreatment.[30] Achong et al.[29] obtained a significant reduction of mutans

streptococci in saliva for 4 months; however, the application of CHX coincided with caries restorative treatment.

Mutans streptococci are harbored in retentive areas of the dentition that are not accessible to currently configured treatments. Several studies have supported the view that the reappearance of mutans streptococci on dentition disinfected with CHX is due to regrowth of organisms that were not eradicated rather than to the introduction of new organisms from outside.[33] Many studies have pointed out the course of regrowth of the mutans streptococci on the inaccessibility of the bacteria to anti-biological drugs. These studies did not consider the biofilm concept. We obtained the sufficient effect of CHX, even by the low concentration of it. We suggest that the combined use of PMTC and anti-bacterial drugs provides an effective preventive system.

This study and our previous studies[21,23] have demonstrated that CHX does not affect the numbers of Lactobacilli and total Streptococci in the oral cavity. As in previous studies with CHX, the total Streptococci levels were not affected by the experimental treatment. The 3DS with CHX is fairly specific in suppressing mutans streptococci in the oral flora, because 3DS tends to make direct contact with the tooth surfaces that primarily harbor mutans streptococci. A side effect associated with 3DS will be minor in nature, since CHX does not come into contact with the oral mucosa. We described in above, the side effect was not admitted, since CHX was applied for only twenty minutes and the concentration was lower than other studies. A single application of low-concentration CHX gel caused a strong initial suppression, but this was of only short duration.[24,34] Long-term suppression of mutans streptococci was achieved by the repeated application of CHX. However, repeated applications, even by individual trays, are sometimes accompanied by side effects such as a burning sensation in the oral mucosa or a change in the taste of food.[28] The absence of side effects with 3DS may be explained by the minimum contact between CHX and the oral mucosa.

The application of CHX by drug retainer appeared to be capable of suppressing the population of mutans streptococci in the total Streptococci even at a low concentration. Application of 3DS combined with PMTC treatment is essential for preventing dental caries. In this study, we used the fluoride together as the home care. In Japan, more than 85% of schoolchildren are using the fluoride toothpaste of 900 ppm or less.[35] The main purpose of fluoride is a remineralization of the tooth. By the in vitro study, it was reported that the fluoride was effective for floating bacteria in saliva.[36] The mechanism of the fluoride is assumed to control the acid secretion by obstructing the enolase revitalization that is the enzyme of the glycolytic system of the mutans streptococci. However, fluoride did not show antimicrobial effect for bacteria in the biofilm. Furthermore, the antimicrobial effect of the fluoride is not clarified in epidemiology. Therefore, in this study, we thought that the factor for controlling effect to the number of bacteria is CHX. The effects of NaF assumed to be little.

In this study, the effect of 3DS using 0.2% CHX combination with PMTC were surveyed in the pilot study of twelve subjects small groups. In spite of the PMTC only group returned to base level, 3DS group kept a low level of the mutans streptococci as compared with total Streptococci at two months later.

It was effective admittedly against the proportion of the mutans streptococci of the total Streptococci, but not significant difference because of small sample size. In power analysis, we calculated the sample size that obtained a significant difference in two groups each study period. We will convince that it is demonstrable the true effect of 3DS with a significant difference for more 30 subjects group, as the further study.

In conclusion, our results indicate that 3DS in combination with PMTC is an effective intraoral drug delivery system that specifically reduce the mutans streptococci, especially in proportion of the total Streptococci without any adverse effect.

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