PEDIATRIC DENTAL JOURNAL 17(1): 1–7, 2007

A randomized controlled trial by the 3DS for dental caries

Seiko Katsumura*[1], Fusao Nishikawara*[2], Yoh Tamaki*[2,3,4], Yoshiki Nakamura*[5], Keita Sato*[6], Yoshiaki Nomura*[2,3] and Nobuhiro Hanada*[2]

*[1] Department of Anatomy, Tsurumi University School of Dental Medicine
2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, JAPAN
*[2] Department of Oral Health, National Institute of Public Health
2-3-6 Minami, Wako, Saitama 351-0197, JAPAN
*[3] Department of Preventive Dentistry and Public Health, Tsurumi University School of Dental Medicine
2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, JAPAN
*[4] Division of Oral Health, Department of Health Science, Kanagawa Dental College
82 Inaoka-cho, Yokosuka, Kanagawa 238-8580, JAPAN
*[5] Department of Orthodontic, Tsurumi University School of Dental Medicine
2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, JAPAN
*[6] Department of Forensic Medicine and Dentistry, Tsurumi University School of Dental Medicine
2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, JAPAN

Abstract The aim of this study is to evaluate the povidone-iodine for the suppression of oral cariogenic bacteria and compare its effects to that of fluoride in children. We carried out a randomized controlled trial to compare the effects of povidone-iodine to those for sodium fluoride. Pre-school children (n = 210) with more than 10^5 cfu/m*l* mutans streptococci in their stimulated saliva were randomly sampled and randomly allocated into three groups. We planned the preventive program by 3DS (Dental Drag Delivery System). The groups that provided preventive programs were the povidone-iodine group (n = 70) and the sodium fluoride group (n = 70). A control group (n = 70) received no intervention. Povidone-iodine or sodium fluoride was applied to the tooth surface using custom made trays. Subsequent home care was obligatory for one month. After one month, the salivary levels of mutans streptococci were low level when compared to the based data. However the difference in salivary levels of mutans streptococci among groups was not statistically significant when comparing the povidone-iodine group with the sodium fluoride group ($P = 0.625$). After 2.5 years follow up, differences in incidence of new dental caries among the three groups were not statistically significant ($P = 0.583$). Clinical application of anti-microbial drugs may be effective, but only a single intervention is not sufficient to reduce the incidence of new dental caries.

Key words
Anti-microbial drugs,
Dental drug delivery system,
Incidence of dental caries,
Mutans streptococci,
Randomized controlled trial

Introduction

The prevalence of dental caries has been declining in most the western countries. This tendency has also been observed in Japan[1)]. Nevertheless, under such conditions subjects with many dental caries are still observed, especially in young children. At age six, permanent teeth begin to erupt. Susceptibility to dental caries is high for premature permanent teeth. At this stage, caries prevention programs may be effective[2)]. Many studies have tested fluoride for prevention[3–5)]. These studies have shown the efficacy of fluoride in improving public health. However, it has been suggested that dental caries are a multi-factorial disease. Mutans streptococci (MS) and Lactobacilli are well known as cariogenic bacteria[6,7)] and these remain as risk factors among pre-school children in Japan[8)]. MS including *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus*

Received on May 16, 2006
Accepted on December 6, 2006

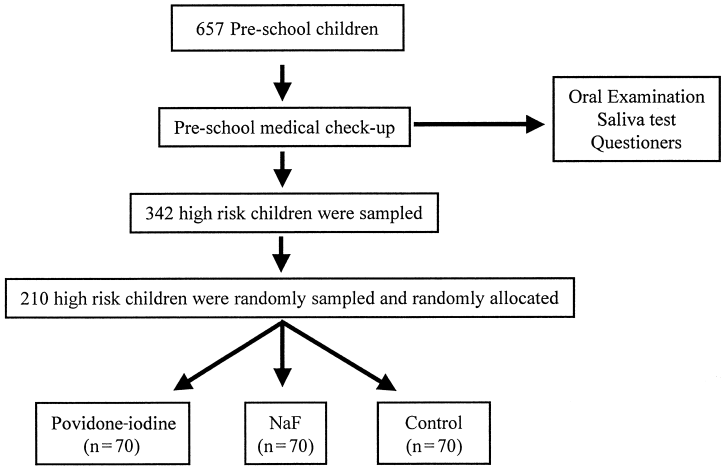

Fig. 1 The study design for children participated in this study

The children that evaluated more than 10^5 cfu/m*l* of MS were designated as high risk children. Twelve children dropped out to participate for the medical check-ups, primarily because of missing check-ups due to illness.

(*S. sobrinus*) are well known groups of oral microorganisms that contain virulence factors that are harbored on tooth surface components as an oral biofilm.

Some reports have shown that anti-microbial drugs can control MS[9–11]. However, few studies are available regarding anti-microbial drugs for prevention in public health[11–13]. Our previous report had shown that these bacteria are suppressed by the Dental Drug Delivery System (3DS)[14]. The 3DS is a risk reduction treatment and a method to control MS in biofilms. It is possible to destroy on the tooth surface using professional mechanical tooth cleaning (P.M.T.C.). The planktonic cells are targets of the anti-microbial drug treatment, which make it easy for the drugs to make contact with the bacteria. This system uses individual trays called drug retainers that apply the anti-microbial drugs onto the dentition immediately after P.M.T.C. The drug retainer minimizes contact of the anti-microbial drugs to the oral mucosa, and optimizes its effect for the tooth surface without dilution by saliva. Our previous report on 3DS using 0.2% Chlorhexidine (CHX) indicated that MS was controlled in adults[15,16]. However, in Japan, use of CHX for the mucosal surface has been prohibited. In contact, povidone-iodine is consequently available for mouth rinse. And its safety has been confirmed[17].

In the current study, we used povidone-iodine as the anti-microbial drugs for the suppression of oral cariogenic bacteria and compared its effect to that of fluoride in children. The incidence of new dental caries was investigated during 2.5 years follow up.

Materials and Methods

Study design and population

Study population was sampled from 657 pre-elementary school children (five or six years old) residing in the Ena-Nakatsugawa area in Gifu prefecture in Japan. The fluoride concentration of drinking water in this area is less than 0.8 ppm. All subjects and their parents gave written informed consent to participate in this study. The children received pre-school medical check-ups including oral examinations and saliva tests. Oral clinical examinations were carried out under a light and dental mirrors were used to explore for caries. At the same time, stimulated saliva samples were obtained following 3 min of chewing gum base that had no taste ingredient or flavor. The details of stimulated saliva sampling were described in our previous report[8]. Children with salivary levels of MS greater than 10^5 cfu/m*l* saliva were designated as high risk for dental caries[18].

From the 342 children at high risk, 210 were randomly sampled and randomly allocated to three groups. One group received povidone-iodine, another NaF and the control group received only a recommendation for treatment of dental caries at a private dental office. The incidence of new dental caries lead been followed arrival check-ups for two years and half. The population and design of this study are shown in Fig. 1.

Table 1 Baseline characteristics of the three groups at preschool medical check-ups

	NaF (n = 48)		Povidone-iodine (n = 54)		Control (n = 69)		Total (n = 171)		
	mean	SD	mean	SD	mean	SD	mean	SD	*P*-value
saliva value (m*l*)	2.84	1.77	2.77	1.40	2.43	1.38	2.68	1.53	0.406
saliva pH	7.28	0.20	7.27	0.20	7.23	0.24	7.26	0.21	0.525
Lactobacilli ($\log_{10}$cfu/m*l*)	4.97	0.72	4.84	0.88	4.77	0.82	4.86	0.81	0.382
total mutans streptococci ($\log_{10}$cfu/m*l*)	6.99	0.26	7.01	0.23	7.03	0.21	7.01	0.23	0.429
MS ($\log_{10}$cfu/m*l*)	6.07	0.26	6.08	0.26	6.10	0.24	6.08	0.25	0.843
ratio of MS in total mutans streptococci (%)	14.95	11.20	14.43	8.78	13.28	7.14	14.22	9.18	0.873
d	2.60	2.97	2.41	2.63	2.04	2.94	2.35	2.85	0.182
f	3.31	3.26	3.46	3.19	5.07	3.81	3.95	3.51	0.001
df	5.91	4.31	5.87	3.66	7.12	4.87	6.30	4.33	0.327

d: decayed teeth, f: fillings in teeth, df: sum of decayed teeth and fillings in teeth
No statistically significant differences were observed for any factors by the Kruskal Wallis test except for fillings.

Microbial procedures

To quantify the salivary levels of total Streptococci, MS and Lactobacilli in saliva, microbial procedures were carried out according to methods described previously[19]. Briefly, 49.2μl of saliva samples were vortexed for ten seconds and inoculated onto Mitis-Salivarius agar (MS: Difco, Tokyo, Japan) medium for total streptococci counts. The modified MSB agar medium[20] contained MS agar (Difco, Tokyo, Japan) supplemented with 20% sucrose (Wako Pure Chemicals Co., Osaka, Japan), 20 mg/m*l* Yeast Extract (Becton Dickinson, MD, USA), 0.25 U Bacitracin (SIGMA, MO, USA), 10 mg/m*l* Colistin (Wako Pure Chemicals Co., Osaka, Japan), 10 mg/m*l* Nalidixic Acid (Wako Pure Chemicals Co., Osaka, Japan), 4 mg/m*l* Gramicidin (SIGMA, MO, USA) and 1% tellurite solution for MS, and Rogosa SL agar medium[21] for Lactobacilli using an EDDY JET spiral system (Gunze Sangyo, Inc., Tokyo, Japan). After anaerobic incubation for 42 h for total Streptococci and MS, and for 72 h for Lactobacilli.

The visible colonies grown on these media were counted using a spiral systems counting grid. Then colony count data were transformed to $\log_{10}$cfu/m*l*.

Clinical examination and dental treatment

After check-ups, children were recommended to visit a private dental office for dental caries treatment. After treatment of dental caries at a private dental office, children belonging to the povidone-iodine and NaF groups were treated with the 3DS program. In this program, the drug retainer was fabricated. After the oral biofilm was removed by P.M.T.C., 10% povidone-iodine (ISODINE GEL: Meijiseika Co., Ltd., Tokyo, Japan) or 950 ppm NaF (Check-up foam: LION Co., Ltd., Tokyo, Japan) was applied to the tooth surface for 5 min using the drug retainer. For follow up home care, 0.45% povidone-iodine (Isodine Nodo Fresh: Meiji Co., Ltd., Tokyo, Japan) or 950 ppm NaF (Check up foam: LION Co., Ltd., Tokyo, Japan) were applied for 5 min using the drug retainer once a day before sleep for one month. After one month, children were invited to the private dental office to take the saliva test.

Statistical analysis

The MS population within total mutans streptococci at check-ups, both before treatment and after treatment, was analyzed using the Wilcoxon signed rank test. Differences between the NaF and povidone-iodine groups were analyzed using the Mann-Whitney U test. Kruskal Wallis tests were used to evaluate differences in baseline characteristics and incidence of new dental caries among these groups. These analyses were carried using SPSS software Ver. 12.0 (SPSS Co., Ltd., Tokyo, Japan).

Results

From the 342 children designated as being at high risk, 210 were randomly sampled and assigned to

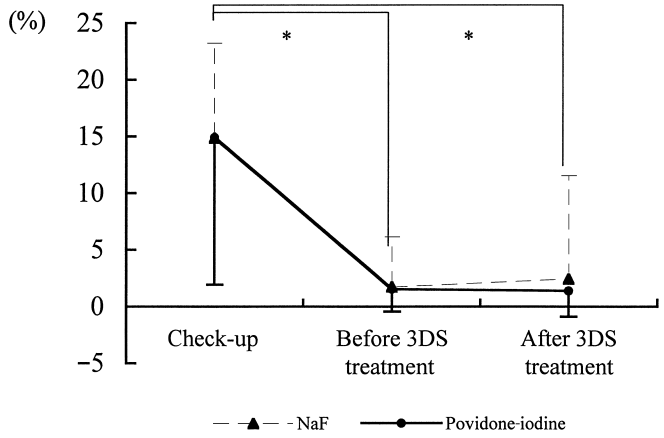

Fig. 2 Proportion of MS in total mutans streptococci before and after 3DS treatment. Data was expressed by mean and SD by each group

* shows the statistically significant difference ($P<0.001$) by Wilcoxon signed rank test. There was no statistically significant difference between NaF group and povidone-iodine group by Mann-Whitney U test ($P=0.625$).

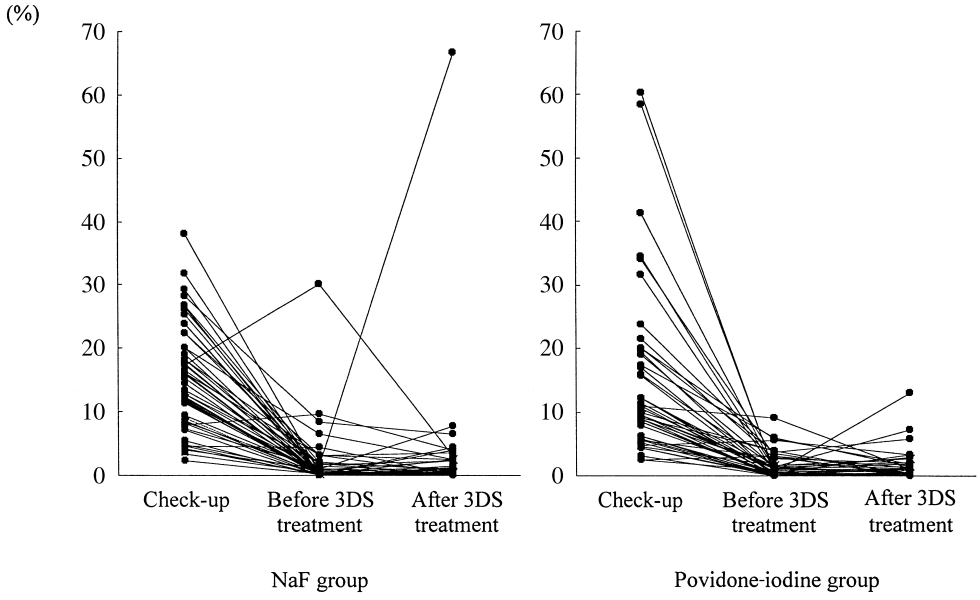

Fig. 3 Individual proportion of MS in total mutans streptococci before and after 3DS treatment

three groups. After sampling, one child dropped out because of relocation. The children belonging to the povidone-iodine or NaF groups received the 3DS as prevention programs at a private dental office. Thirteen children refused to receive the prevention programs. And three children in the povidone-iodine group and 11 in NaF group, respectively, did not go to the private dental office. The number of subjects taking the preventive programs was 54 in the povidone-iodine group and 48 in the NaF group. One child in each group dropped out to take the saliva test after the preventive programs.

The characteristics of the three groups are shown in Table 1. No statistically significant differences in these factors were found except for the number of fillings teeth. The changes in the proportion of MS in total mutans streptococci before and after treatment are shown in Fig. 2, and the individual proportions of MS in total mutans streptococci are shown in Fig. 3. The MS population was decreased dramatically before treatment compared with medical check up time in both povidone-iodine and fluoride groups

Table 2 DMF increments over 2.5 years of follow up in each group

		NaF		Povidone-iodine		Control		Total		
age		mean	SD	mean	SD	mean	SD	mean	SD	*P*-value
6Y		n = 50		n = 45		n = 66		n = 161		
	D	0.00	0.00	0.07	0.33	0.05	0.27	0.04	0.27	0.360
	M	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.000
	F	0.00	0.00	0.02	0.15	0.08	0.44	0.04	0.29	0.480
	DMF	0.00	0.00	0.09	0.36	0.12	0.51	0.07	0.38	0.191
7Y		n = 47		n = 37		n = 50		n = 134		
	D	0.09	0.35	0.19	0.74	0.08	0.34	0.11	0.49	0.911
	M	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.000
	F	0.02	0.15	0.05	0.23	0.16	0.62	0.08	0.41	0.270
	DMF	0.11	0.38	0.24	0.76	0.24	0.69	0.62	0.55	0.562
8Y		n = 49		n = 42		n = 64		n = 155		
	D	0.18	0.57	0.21	0.78	0.20	0.51	0.20	0.64	0.638
	M	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.000
	F	0.12	0.39	0.02	0.15	0.11	0.44	0.09	0.37	0.330
	DMF	0.31	0.65	0.24	0.79	0.31	0.83	0.29	0.76	0.584

D: decayed teeth, M: missing teeth, F: filling teeth, DMF: sum of decayed teeth, missing and filling teeth, Y: years old
There was no statistically significant difference in each group by Kruskal Wallis test.

($P<0.001$). However, the difference between the groups of the changes in MS was not statistically ($P=0.625$).

Follow-up data of one to 2.5 years indicated that there were no statistically significant differences. In Japan, these medical check-ups including the oral examination are obligated by law for school children. Data regarding oral health of school children are obtained through these check-ups every year. The D, M, F and DMF increments over 2.5 years for each group are shown in Table 2. After 2.5 years of observation, no statistically significant differences regarding the incidence of new dental caries were observed ($P=0.583$).

Discussion

MS adheres to tooth surface and becomes a constitutive element for biofilm, so it is known as a cariogenic bacterium. Some studies had shown that the clinical availability of anti-microbial drugs for the reduction of MS[9,12,13] subsequently suppressed the incidence of new dental caries[22].

CHX was used representatively as the anti-microbial drug for prevention of dental caries[23,24]. CHX had a high antibacterial effect against MS[9,25–27]. Moreover, it allows a high concentration of CHX to be applied on the tooth surface, for example, via varnish or gel applications[28,29]. However, anti-microbial drugs cannot infiltrate the deep part of the biofilm[30] because of their molecular weight. Treatments using only anti-microbial drugs have been discussed and appear to be ineffective for treatment of biofilm. Therefore, biofilms should be removed by mechanical procedures and then chemical treatments against the small amount of remaining planktonic cells should be performed to prevent re-adherence of MS. This means that cariogenic bacteria are removed from tooth surfaces, and replaced by indigenous bacterial flora that are not cariogenic.

So we recommend 3DS: to apply the anti-microbial drugs to tooth surfaces using the drug retainer and after removing the biofilm by P.M.C.T.[10,15]. Our previous report showed that the availability of programs suppressed salivary levels of MS for three months[10]. The study population consisted of adults and the observation periods were very short. Additionally, our previous report had shown that high concentrations of CHX suppress salivary levels of MS[16]. However, CHX has side effects such as taste abnormalities, burning sensations

of the oral mucosa, anaphylactic shock, unpleasant tastes, and staining of the teeth[31,32]. Therefore, CHX is contraindicated for treatment of the oral mucosa and regulated for use in Japan. Consequentially, povidone-iodine as the anti-microbial drug has been used for the prevention of early childhood caries.

We evaluated cariogenic bacteria at three points: at check-ups, before the prevention program (after the dental treatment), and after the preventive program. A drastic reduction in MS was observed after treatment of dental caries. This may be because of the inhabitation of MS in the dental caries cavity. These bacteria may be released from the saliva stimulated by chewing gum. As a result of treatment of dental caries, it is conceivable that MS in saliva is drastically reduced. However, no statistically significant difference was observed between the povidone-iodine and NaF groups. Both two groups the MS in saliva kept the low level, but the reductions were not statistically significant after the preventive program either. And after 2.5 years follow up, no statistically significant differences in the incidence of new dental caries were observed among the three groups. It seems that to reduce the incidence of new dental caries via reducing MS, a one-time intervention of povidone-iodine is not sufficient. The results of our study are in agreement with a previous study about the effect of povidone-iodine in children[33]. They also applied CHX, and cariogenic bacterial levels of the both eventually increased to pretreatment levels[23,24,30].

For caries prevention, fluoride has generally been used[5]. The important action of fluoride is the enrichment by stabilization of hard tissue structures[34]; the antibacterial effect of fluoride is not recognized[35]. MS are highly adherent to tooth surfaces, and live selectively on tooth surfaces. Povidone-iodine has a strong antibacterial effect. Therefore, it is expected to have a bacteriostatic effect on the oral cavity. Povidone-iodine is able to affect the oral mucosa and in generally causes no adverse effects compared with CHX[13]. The adverse such as occurs very limited cases.

Thus, in Japan, under the limited conditions for the anti-microbial usage, we considered povidone-iodine as a suitable anti-microbial drug, especially for children. Continuous use of povidone-iodine is necessary in practical use. Further study is now necessary to elucidate the effect of povidone-iodine on oral microflora and to pursue the mechanism by which this drug prevents dental caries, especially regrowth of MS. We conclude that only a single intervention is not sufficient to reduce the incidence of new dental caries. Therefore, it is most important to control cariogenic bacteria by regular interventions.

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