Antibacterial Treatment Needed for Severe Early Childhood Caries

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Abstract

Objectives: *To assess the effects of a single 10% povidone iodine application as an adjunct to extensive surgical procedures in the clinical treatment of children with early childhood caries.* ***Methods:*** *Twenty-two children scheduled for dental treatment under general anesthesia were randomized into either an intervention group (10% povidone iodine), or a control group (phosphate buffered saline). Either povidone iodine or phosphate buffered saline was applied to teeth and soft tissues after prophylaxis and all operative dental procedures, followed by1.23% acidulated phosphate fluoride gel. Saliva samples taken at baseline, and after 1 hour, 3 weeks and 3 months were assayed for mutans streptococci, lactobacilli and total viable bacteria. Caries lesions were recorded at baseline and at one year.* ***Results:*** *Mutans streptococci and lactobacilli levels in the povidone iodine group were significantly reduced relative to baseline at 1 hour, 3 weeks and 3 months. At one year at least 60% of subjects had new caries lesions in each group, and there was no significant difference in caries increment between the two groups.* ***Conclusions:*** *Even prophylaxis, fluoride gel application and complete surgical treatment of caries at baseline were insufficient to prevent new caries in over 60% of the patients in these high caries risk infants. Although the one-time treatment with povidone iodine reduced mutans streptococci and lactobacilli levels for up to 3 months this therapy failed to additionally reduce future caries formation over one year, indicating that repeated antibacterial treatments will be needed to control high levels of cariogenic bacteria.*

Key Words: Povidone Iodine, caries, mutans streptococcus, lactobacillus, antibacterial treatment

Introduction

Early childhood caries (ECC) is a major public health problem in the US, and indeed worldwide. A comprehensive review of the epidemiology of ECC shows that its prevalence varies from population to population(1); disadvantaged children, regardless of race, ethnicity or culture are most vulnerable, and this earlier report is substantiated by several recent articles as reviewed recently by Den Besten and Berkowitz (2). This form of dental caries is of great concern when it is serious enough for infants and young preschool children to require treatment under general anesthesia. In such cases extensive restorative work is done in the operating room and the costs are high (2). The current standard of care for ECC is usually restricted to surgical removal or restoration of carious teeth, application of topical fluoride, oral hygiene instructions and recommendations regarding feeding behaviors. Dental surgery has minimal impact on oral MS reservoirs (3,4). Clinical outcomes for treatment of ECC are poor; nearly 40% of children treated for ECC, even with restorative work done under general anesthesia, have been shown to develop new caries lesions within 6-12 months post-dental surgery (3,5-8).

Dental caries is an infectious disease. Its initiation and progression depend on a balance between demineralization and remineralization of the enamel (23). Numerous microbiological studies have shown positive correlation between heavy *mutans streptococci* (MS) and *lactobacilli* (LB) infections and caries in children (9-16). Results from studies (10,17,18) also demonstrated that in children with severe early childhood caries, *mutans streptococci* regularly exceeded 30% of the cultivable plaque flora. Conversely, *mutans streptococci* typically comprise less than 0.01% of the plaque flora in children with negligible to no caries activity.

These previous studies indicate that meticulous surgical treatment alone is insufficient and it seems likely that improved clinical outcomes for ECC may be realized by recognizing the infectious nature of this disease and incorporating antibacterial therapy as a part of caries prevention and treatment. Iodine has received some attention for many years as a potential antibacterial for anticaries therapy. In early studies topical use of iodine showed prolonged suppressive effects on oral populations of *mutans streptococci* (24). A recent small clinical study by Lopez *et al.* in Puerto Rico showed that bimonthly topical application of a 10% povidone iodine solution (1% active iodine) to the teeth of infants at high risk for ECC inhibited the development of white spot lesions (21, 22). However, no bacterial results were reported. This very promising result

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warrants further study, since if povidone iodine can be used as a successful caries antibacterial it will provide a means for enhancing caries reductions in both individual and in public health settings.

The current standard of care used by Pediatric Dentistry at UCSF for severe caries children in the operating room includes a thorough prophylaxis, topical fluoride application and meticulous surgical procedures. The objective of the present study was to assess the results of using a one time 10% povidone iodine application as an adjunct to the clinical treatment of infants with severe early childhood caries who required extensive surgical procedures under general anesthesia. The hypothesis to be tested was that a one time application of povidone iodine is an effective antibacterial agent against cariogenic bacteria, and that bacterial reductions would be manifested as improved caries prevention.

Methods and Materials

Subject recruitment, randomization and blinding. Twenty-two children scheduled for dental treatment under general anesthesia (GA) at the UCSF Pediatric Dental Clinic were recruited in this pilot study. The study protocol was approved by the UCSF Committee on Human Research. Informed consent was obtained from the parents/guardians of the eligible subjects after explaining the aim and procedures of the study. Inclusion criteria were as follows: 1) generally healthy children with active caries in at least four of the primary incisors and canines; 2) age range from two to six years; and 3) scheduled for dental treatment under general anesthesia. Exclusion criteria were: 1) systemic diseases or periodontal disease; and 2) antibiotics or medications that might affect oral flora or saliva flow taken within the previous 3 months. Following the clinical examination (described below) the consenting subjects were randomized to either the intervention (10% povidone iodine, 11 subjects) or control (phosphate buffered saline, 11 subjects) groups. Only the clinician administering the povidone iodine or control treatment (PKDB) and the investigator in charge of the study (LZ) knew which subjects were in each group. The subjects, parents/guardians, the pediatric dentist responsible for the caries examination, and laboratory personnel responsible for the microbiological assays were blinded to the test or control treatment that the subjects received. All samples were labeled and coded only with study identification numbers to ensure blinding and all laboratory procedures and calculations were conducted blinded.

Clinical procedures. After anesthesia was induced, a baseline saliva sample was obtained from each subject by swabbing the dentition and oral mucosa prior to dental restorative treatment. The swab was immediately placed into a pre-labeled/preweighed tube with 2 ml of phosphate buffered saline (PBS). According to the standard clinical procedures already in place all subjects received a thorough dental prophylaxis prior to the commencement of operative procedures. After the completion of all restorative therapy, another dental prophylaxis was done in all subjects. Subsequently, the intervention group subjects received a 2-minute application of 2 ml of 10% povidone iodine (PI, 1% active iodine, Allegiance Health Corporation, USA) by swabbing the solution on all teeth and mucosal surfaces. Control subjects had 2 ml of PBS applied with the same technique. Excessive PI or PBS was removed by suction. Then a 1.23% acidulated phosphate fluoride (APF) gel (Oral B, USA) was applied to the teeth for 2 minutes in all subjects prior to extubation. This APF treatment is part of the normal standard of care at UCSF and was required to be included in the treatment for all patients. Another swab sample for bacterial analysis was taken approximately 1 hour after recovery from anesthesia. Two additional visits were then scheduled for collection of swab/saliva samples 3 weeks and 3 months after the dental treatment. All swab/saliva samples were cultured for bacterial enumeration as described below. The presence of potential side effects, including tooth staining, taste change and allergic reactions were recorded at each subsequent visit.

The number of decayed, missing due to extraction and filled teeth (deft) and surfaces (defs) were recorded by the dentist responsible for dental restorative treatment based on bitewing x-rays and a visual examination under GA after a thorough prophylaxis at baseline. These caries exams were conducted prior to randomization and assignment to test or control treatment to ensure blinding. The deft and defs scores were recorded again at 1 year follow-up based on bitewing x-rays and a visual examination in the pediatric clinic by one dentist (TF) who was trained in the pediatric dentistry specialty program at UCSF, and was also responsible for the baseline examinations and participated in the clinical restorative procedures. She was blinded to group assignments. The decayed surfaces or recurrent decayed surfaces were recorded as new ds at 1 year follow-up. Decayed surfaces (ds) or teeth (dt) were recorded only when cavitations were detected. White spot lesions were not recorded. Caries on smooth surfaces were recorded as ss-ds (smooth surface decayed surfaces). Because none of the subjects had filled or missing (extracted) teeth before treatment at baseline, only ds and ss-ds were analyzed in the study. None of the subjects had any restorative treatment between baseline and the one year examination. Therefore the study simply examined the number of new lesions between baseline and one year, with no confounding restorative work before baseline or between baseline and the one year exam.

Restorative procedures were conducted as needed under general anesthesia during one visit using stainless steel crowns, resin restorations, amalgams, extractions, pulpotomies, pulpectomies, and sealants. There were no statistically significant differences between the two groups with respect to any of these materials or procedures.

Swab/saliva sample processing and microbiology. Each tube contain-

Table 1
Mutans streptococci (MS), lactobacilli (LB) and total viable bacteria counts (TVC) as log_{10} CFU/ml (colony forming units) levels at baseline, 1 hour, 3 weeks and 3 months after treatment in both groups

	MS (log_{10} CFU/ml mean and SD)				LB (log_{10} CFU/ml mean and SD)				TVC (log_{10} CFU/ml mean and SD)			
	baseline	1hr.	3 wks.	3mos.	baseline	1hr.	3 wks.	3mos.	baseline	1hr.	3 wks.	3mos.
Intervention group – Povidone iodine												
mean	6.4	0.7**	4.7**	5.2**	4.0	0.5**	1.0**	1.0**	8.5	4.1**+	7.8**	8.2
SD	1.2	1.3	0.7	1.2	1.8	1.1	1.5	1.6	0.6	0.9	0.4	0.9
Control group												
mean	6.2	1.0**	5.3	5.8	3.7	1.2**	1.0**	2.5	8.4	5.6**	8.3	8.3
SD	1.7	1.6	2.1	1.5	2.2	1.5	1.8	2.3	0.7	1.0	0.8	0.6

** significantly different from baseline ($p < 0.01$)
+ significantly different from control ($p<0.01$)

ing a swab/saliva sample was stored on ice until it was transported within 2 hours to the microbiology laboratory at UCSF for bacterial culture. The weight of the tube with the swab/saliva sample was measured and recorded. The sample was then vortexed for 30 seconds and the resulting bacterial suspension was serially diluted with PBS at 10^{-1}, 10^{-3}, and 10^{-5}. An aliquot (0.1 ml) of each dilution was plated on Mitis Salivarius Sucrose Bacitracin agar (MSSB) for mutans streptococci (MS), on tomato juice-Rogosa agar for lactobacillus species (LB), and on BHI agar containing 5% sheep blood for total viable bacteria (TVC). Plates were incubated in 85% N_2, 10% H_2, & 5% CO_2 at 37°C for 3-5 days before enumeration of colonies (as colony forming units (CFU) per ml of saliva).

After the samples were plated, the swabs were dried, and then weighed. The saliva volume collected in the swab was estimated using the following formula:

Volume of the saliva in swab (ml) = (weight of the tube with swab – weight of the pre-weighed tube - weight of the dried swab obtained after sampling) x 1 ml/g.

Statistical analysis. Colonization levels of MS, LB, and TVC (CFU/ml saliva) were determined for each time point as the microbiological outcome measures. For each subject the reductions in log_{10}MS and log_{10}LB were calculated by comparing values from the later visits with the baseline levels. The means and standard deviations of the changes in log_{10} value were calculated for the intervention and control treatment groups and comparisons were made between the two groups at baseline, 1 hour, 3 weeks and 3 month follow-up by 2 sample Student t tests. Comparisons were also made within each group to judge the short term and long term impact of treatment on TVC and cariogenic bacterial recolonization by paired t-tests. Linear regression (analysis of covariance) models with change from baseline to 3 months in log_{10}MS, log_{10}LB counts, and log_{10}TVC as response and ss-ds (baseline values) and treatment group as predictors were performed to compare ss-ds adjusted changes between the treatment groups. Moreover, mixed effects regression models with random person effects were used to examine changes over time in the microbiological data between the two treatment groups while accounting for within-person correlation.

The Student t-test was used to compare ds and ss-ds at baseline and new ds and ss-ds at the one year follow-up between the two groups.

Results

Subject demographics. Twenty-two children were recruited into the study. After randomization and treatment 1 participant was excluded for not meeting the entry age criterion – this person was replaced so a total of 22 subjects enrolled in the study at baseline with 11 subjects in each group. The ethnicity breakdown was 1 Black, 2 White, 2 American Indian, 6 Asian and 11 Hispanic. Eighteen of the subjects finished the 3-month segment of the microbiological study (1 subject in the intervention group and 3 subjects in the control group were lost-to-follow-up at the scheduled 1-month and 3-month visits). Nineteen of 22 subjects completed the 1 year follow-up visit (9 in PI and 10 in PBS). At baseline, the mean age was 3.6 and 3.9 years in the intervention and control groups, respectively.

Microbiology data at baseline and the follow-up visit. The mean log_{10} value and standard deviation (SD) of bacterial counts at each sampling time in both groups are summarized in Table 1. At baseline, all subjects had mutans streptococci (MS) levels over 10^3 CFU/ml with 82% of the subjects (18 of 22 subjects) over 10^5 CFU/ml. Eighty-six percent of the subjects had detectable lactobacilli (LB) with 73% of them (16 of 22 subjects) having LB level over 10^3 CFU/ml. No significant correlations were found between MS, LB or TVC levels and ds or ss-ds scores (Pearson correlation). There were no statistically significant differences in MS, LB and TVC levels found between the two groups at baseline (Table 1, Student t-test).

MS and LB levels in the iodine group were significantly reduced at 1 hour, 3 weeks, and 3 months compared to baseline ($p\leq0.001$, paired t-tests). However, only MS and LB at 1 hour, and LB at 3 weeks were significantly reduced in the control group. Total viable bacteria counts (TVC) were initially significantly reduced but returned to baseline in the control group by 3 weeks and the iodine group by 3 months. Although the mean reductions of log_{10}MS and log_{10}LB at 1 hour, 3 weeks and 3

Figure 1
Reduction in logMS (mutans streptococci) values from baseline at follow-up visits in both groups (Error bars are 1 standard deviation)

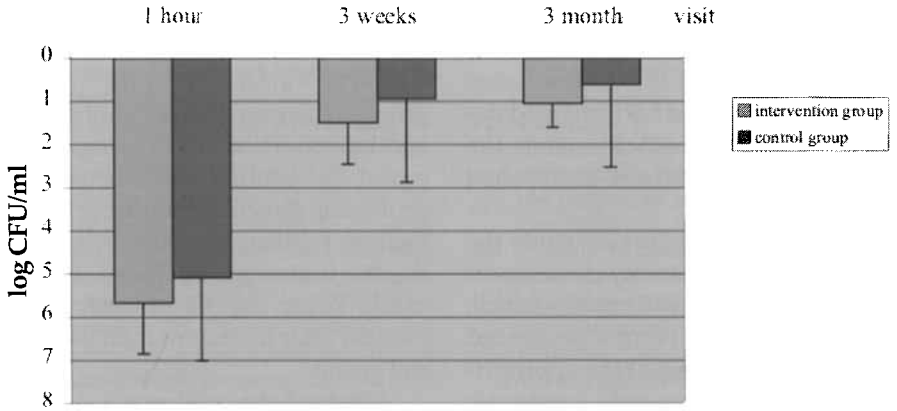


Figure 2
Reduction in logLB (lactobacilli) values from baseline at follow-up visit in both groups (Error bars are 1 standard deviation)

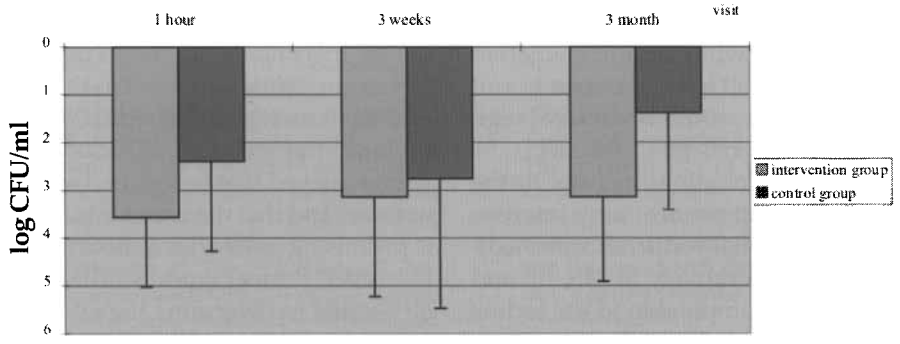
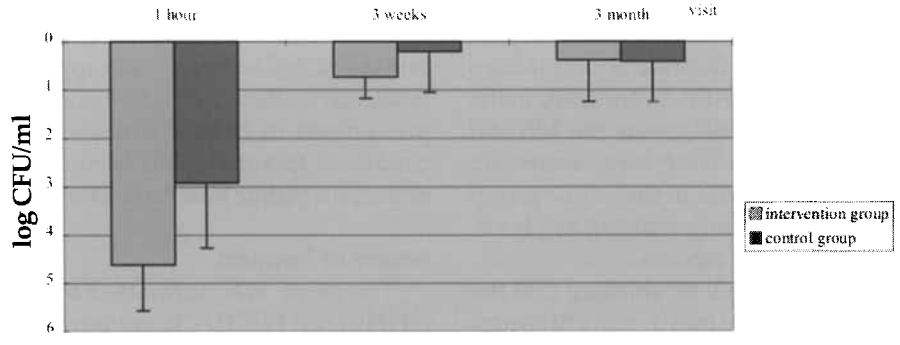


Figure 3
Reduction in logTCV (total viable bacteria counts) values from baseline at follow-up visit in both groups (Error bars are 1 standard deviations).



months in the intervention group were numerically larger than those in the control group, there were no statistically significant differences between the two groups (Figures 1 & 2). Reduction of $\log_{10}$LB at 3 months was significantly correlated to ss-ds (Pearson r=0.686, p=0.002). Adjusting for ss-ds, the intervention group had a significantly larger $\log_{10}$LB reduction at 3 months after treatment than the control group (regression model coefficient=1.74, p=0.01). No statistically significant correlations were found between ds and reduction of $\log_{10}$MS and $\log_{10}$LB. There was a significantly greater reduction in $\log_{10}$TVC in the intervention group at 1 hour after treatment than the control group (student t-test, p= 0.003), the difference in TVC between the two groups was diminished at 3 weeks (p=0.09), and by 3 months had returned to baseline levels (Figure 3).

No tooth staining, complaints of taste change or allergy were observed in either the intervention or control groups. There was no significant post baseline correlation between bacterial levels in either group to either type of restorative material (stainless steel crowns, composite or amalgam restorations) or the restoration or extraction of anterior incisors.

Decayed surfaces at baseline and 1 year follow-up. At baseline, the mean ± SD number of ds and ss-ds were 34.0 ± 10.0, 26.2 ± 9.5 (iodine) and 34.5 ± 10.2, 26.1 ± 10.0 (control) respectively. There were no statistically significant differences in age, ds and ss-ds at baseline between the intervention and control group (Student t-tests, p>0.90).

Nineteen of 22 subjects completed the 1 year follow-up visit (9 in iodine and 10 in control), 3 of 9 (33%) in iodine and 4 of 10 (40%) in control remained caries free at 1 year follow-up. The mean ± SD number of new ds was 4.4 ± 5.3 for PI and 5.0 ± 7.8 for PBS. There was no significant difference in new caries formation at 1 year follow-up between the two groups (Student t-test, P=0.86).

Discussion

In the present study, even with very thorough double prophylaxis and the application of acidulated phosphate fluoride in combination with the surgical procedures in the operating room, only 40% of the subjects in the control group were caries free after one year. 60% had new decay, with five surfaces on average newly decayed. This result is entirely consistent with previous reports from other investigators where 40% had new decay within 6 months post dental surgery (2,3, 6-8. 19, 20). This result alone has major public health implications. It is clear that meticulous cleaning, high concentration fluoride treatment and removal of all decay clinically is not sufficient to halt the progress of severe early childhood dental caries (ECC) in these very

high risk individuals. As shown in the present study, the bacterial infection rapidly recurs, with recolonization to original levels within a month. Thus additional therapy over and above high concentration fluoride is necessary, not just with children destined for treatment in the operating room but for the entire ECC prone population who have not yet reached that serious stage.

The promising results of Lopez *et al. (21,22)* who showed caries reduction over 12 months with bimonthly povidone iodine applications were not borne out in the present study. However, the subjects in the Lopez *et al.* study were caries free at their time of enrollment, whereas the subjects in the present study had severe ECC. The present study, however, did show that significant reductions in cariogenic bacteria with the iodine treatment were maintained for 3 months, but these reductions were insufficient to be manifested as reductions in new caries after 12 months. Similarly to the control group, the PI group also showed new caries lesions after one year in 67% of the subjects, with only 33% remaining caries free. There was no significant difference in new caries levels at one year between the iodine and control groups. It is possible that if frequent application of PI was made the reduction in cariogenic bacterial levels would be maintained and this should translate into lower caries incidence, but this needs to be determined by further clinical studies.

What can be learned from the microbiological results that can indicate future directions for likely success? All subjects in this study had extensive caries experience and treatment under general anesthesia was necessary. Their levels of cariogenic bacteria were very high. These results support previous reports of high levels of cariogenic bacteria infection in children with ECC. If we look at the results for the PI group in isolation they are promising. Immediately postoperatively the levels of MS and LB were low, although not eliminated (Table 1). After 3 weeks they were still significantly depressed compared to baseline. Of particular note is the low value for LB, which remained very low even at 3 months. Also of note are the trends with total bacteria levels (TVC) (Fig 3). By 3 months the TVC values were close to baseline, even though the MS and LB remained depressed, which indicates that the "good" bacteria have re-established at baseline levels.

In the Lopez *et al.*(21,22) study the investigators fortuitously chose repeat treatment intervals of 2 months which, according to the results presented here, may have been at the appropriate time to again lower the cariogenic bacterial levels. This suggests that bimonthly, or even monthly reapplication of povidone iodine may be desirable to control the cariogenic bacteria reemergence. Only a future clinical study combined with microbiological assessments will determine whether that speculation is correct and whether that would be an ideal regimen for high risk ECC patients.

The microbiology results in the control group are also very interesting. The initial reductions immediately postoperatively in MS, LB and TVC were comparable to the iodine group (Table 1, Figs 1-3). However, by three weeks the MS levels were not significantly different from baseline, and at 3 months the LB levels were not significantly different from baseline. It is possible that there was an antibacterial effect in some subjects from the high fluoride concentration applied as an APF. In contrast, in the povidone iodine group, the MS and LB reductions were very consistent. MS and LB levels in the iodine group were significantly reduced at 1 hour, 3 weeks and 3 months.

It is also well established that the APF treatment enhances remineralization and repair of early lesions, but this effect was insufficient to overcome the severe caries challenge in these patients over subsequent months with 67% showing new caries after one year. When the MS and LB data were compared for the PI and control groups the very promising significant reductions (compared to baseline) in MS and LB shown in the PI group, although numerically very different from the control group, were not statistically significantly different from the lesser reductions in the control group at 3 weeks and 3 months. This can be accounted for by the large variability in the control group. However, this does not diminish the results in the PI group compared to baseline, with respect to promising direction for further investigation. Further, after adjusting for ssds, the iodine group had a significantly larger $\log_{10}$LB reduction at 3 months after treatment than the control group.

Although the concept of using antibacterial therapy seems reasonable, it has not been widely used in the prevention of dental caries. There are no specific antibiotics or vaccines yet available against cariogenic bacteria. The present study showed, that under the meticulous conditions that the povidone iodine was used in the operating room significant reductions in mutans streptococci and lactobacilli were achieved that lasted for 3 weeks or more, and that the use of this agent is promising as a caries antibacterial. However, future clinical studies will be needed to determine the effect of frequent povidine iodine applications on cariogenic bacterial reduction, and whether this will translate into reductions in caries increments. If the use of frequent applications of antibacterial treatments such as povidone iodine, coupled with enhancement of remineralization by fluoride applications, can be shown effective in caries prevention in future studies such combined therapy could become an important public health measure.

Source of Support

The study was supported by US DHHS/NIH/NIDCR cooperative agreement 1-U54-DE14251 and the DentiCal Plan of California.

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