Original Article

Braz J Oral Sci.
January | March 2011 - Volume 10, Number 1

Effect of chlorhexidine gel containing saccharin or aspartame in deaf children highly infected with *mutans streptococci*

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Abstract

Aim: Since deaf children are unable to comprehend or cooperate with dental treatment due to lack of communication, preventive measures may be an important way to control the high prevalence of dental caries in these patients. The aim of the this study was to evaluate the effect of an intensive treatment with chlorhexidine (CHX) gel, containing either saccharin or aspartame, in deaf children highly infected with mutans streptococci (MS). **Methods:** Eighteen children were randomly divided into two groups, according to the sweetener used to improve the CHX gel bitter taste: saccharin or aspartame. Before CHX treatment, saliva samples were collected to establish baseline microbial data for MS. CHX gel was applied on two consecutive days, four times the first day and three times the second day. Saliva samples were then taken after 7, 30, 60, 90 and 120 days to evaluate MS oral recolonization. **Results:** CHX gel containing saccharin was not effective on the reduction of MS levels, while the gel containing aspartame decreased significantly MS levels after treatment ($P<.05$). **Conclusions:** Although a new CHX application may be necessary after 60 days to control caries risk and MS levels, CHX treatment should be individually controlled because of variations in the response of subjects.

Keywords: chlorhexidine gel, saccharin, aspartame, *mutans Streptococci,* saliva, deaf children

Introduction

Dental caries results from the interaction of specific bacteria with constituents of the diet within a biofilm termed 'dental plaque'[1]. Among the cariogenic bacteria, mutans streptococci (MS) are most closely associated with the development of caries disease[2-3] and, therefore, salivary MS levels have been used as an indicator of caries risk[4-5]. Therefore, one way of controlling cariogenic activity would be to reduce the MS oral levels by the use of antimicrobial agents[6-7].

Among the antimicrobial agents available for dental use, chlorhexidine (CHX) is the most effective and most widely documented substance in controlling cariogenic activity[6,8-10]. CHX reduces the proportions of some microorganisms, especially MS, which are particularly sensitive to this substance[4,11-13]. The major

Received for publication: May 12, 2010
Accepted: December 20, 2011

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advantage of CHX over most other compounds lies on its oral substantivity[14], as it is a cationic substance that adsorbs to soft and hard tissues of the mouth[15] as well as to bacterial cell walls[16]. Because CHX has an extremely bitter taste, it is often necessary to flavor and sweeten gel products. However, when formulations are prepared the availability of CHX can be impaired. Regarding this, there are few studies[17] evaluating the effect of sweeteners commonly used to improve the gel taste on the antimicrobial activity of CHX.

Studies with highly MS-infected children have demonstrated that an accentuated reduction of MS levels occurs after an intensive treatment with CHX gel[13,18-19], with MS levels returning gradually to those verified initially within 2 to 6 months after treatment[6,20-21]. However, there is no report on the effect of this antimicrobial agent in subjects with disabilities, such as deaf children. For special needs patients, the use of CHX gel may be an important method to control the high prevalence of caries disease and poor oral hygiene[22-23], since dental care is often neglected in those children because of poor cooperation with dental treatment due to lack of communication[24]. Thus, the aims of this study were: (a) to compare the effect of intensive treatment with CHX gel, containing saccharin or aspartame, in decreasing the MS levels in deaf children and (b) to evaluate the reappearance pattern of MS over time after the CHX applications in those subjects.

Material and methods

Experimental design

This prospective study involved a randomized, double-blinded (regarding patients and examiners) design approved by the Research Ethics Committee of the University Hospital of the Federal University of Maranhão (Protocol n.º 33104-00320/2002). The parents or the children's legal tutors read and signed an informed consent form, in which all procedures, possible discomforts or risks, and benefits were fully explained. Eighteen patients, aged 5-10 years, who fulfilled the inclusion criteria were selected from a special school for deaf children, located at the city of São Luis, in the northeast of Brazil. Inclusion criteria involved healthy deaf children of both genders, highly infected with MS (1.0×10^6 CFU mL^{-1} of saliva)[4-5]. Exclusion criteria excluded those using any type of antimicrobials or even chemical products for mouthrinsing or topical use, during a minimum interval of 6 months before the beginning of the experiment. All children received restorative dental care at the public dental health service prior to the start of the experiment.

Children were randomly divided into two groups, according to the sweetener used to improve the CHX gel bitter taste: saccharin (n = 9) or aspartame (n = 9). The number of subjects in each test group was determined by comprehensive preliminary tests that demonstrated that the sample size yielded an adequate power (0.80) to detect statistically significant differences. The treatment with 1% CHX gel was performed according to the protocol established by Maltz et al. (1981)[18]. Before CHX treatment, saliva samples were collected to establish baseline microbial data for MS. After the application of CHX, saliva samples were taken in the following periods: 7, 30, 60, 90 and 120 days to evaluate MS oral recolonization. Microbiological analysis of the saliva was made using a dip slide test for MS.

Saliva sample collection

Children underwent the saliva sample collection between 9.30 and 11.00 a.m., at least 2 h after breakfast and before the mid-morning snack. Then, a paraffin-stimulated saliva sample of approximately 2.0 mL was collected. The saliva produced in the first 30 s was discarded, beginning the collection after this time. The saliva was picked up in sterile test tubes, containing 10 glass pearls. The tube was conserved in ice[25].

Microbiological analysis of the saliva

Saliva samples were microbiologically analyzed using a dip slide test for MS, CARITEST-SM (Herpo; Dental Products Ltda, Rio de Janeiro, RJ, Brazil), which is similar to the test proposed by Jordan et al. (1987)[26], marketed commercially as CARIESCREEN SM (Automated Diagnostic Documentation, Grand Haven, MI, USA).

The test tubes containing the saliva samples were homogenized individually in a vortex mixer, during 1 min. Soon after, using an automatic micropipette with sterile plastic tips, 1.5 mL was poured into the tube with buffer solution and bacitracin previously added. This tube was slightly agitated and the dip slide was immersed in it. Meanwhile, a CO_2 generating tablet was placed into the empty tube with 2 drops of distilled water. The dip slide was immediately removed from the solution and immersed in the tube with CO_2, which was sealed. All tubes containing the dip slides were incubated at 37° C for 48 h and remained at controlled room temperature at 23° C for 24 h.

A single examiner underwent a single pre-study calibration session to be familiar with the codes for converting the appearance of the incubated dip slide to an approximation of the number of colony-forming units per mL of saliva (CFU mL^{-1} of saliva). The colony density chart was supplied by the manufacturer, which showed 6 CFU scores: 10,000; 50,000; 100,000; 250,000; 500,000 and 1,000,000 CFU mL^{-1} of saliva. In terms of bacterial count, children with an MS level e" 100,000 CFU mL^{-1} of saliva were classified as high caries risk[4-5].

Application of CHX

The 1% CHX gel (Facial Pharmacy, São Luís, MA, Brazil) was prepared containing saccharin or aspartame as sweetener to improve the CHX gel bitter taste. Prior to the CHX application, children received reinforcement of basic oral hygiene, and the parents or the children's their legal tutors were instructed to restrict the children's consumption of sucrose. After professional prophylaxis, a disposable tray was used for CHX application, in accordance with the protocol established by Maltz et al. (1981)[18] – first day with four 5-min applications and 5-min intervals between each

application; and second day (24 h after) with three 5-min applications and 5-min intervals between each application. During these intervals, the children mouthrinsed with water, and the trays were prepared for the next application.

Statistical analysis

The non-parametric Wilcoxon test was applied to the intragroup data. Statistical analysis was done using BioEstat software (version 3.0, 2003; Brazil) with the significance level set at 5%.

Results

The results obtained after the application of the CHX gel containing saccharin can be observed in Table 1. The CHX gel was not effective on the reduction of MS levels in saliva, as no statistically significant difference (Wilcoxon test, $p>.05$) was observed between the data presented after and before CHX application (1.0×10^6 CFU mL^{-1} of saliva). It was verified that the MS levels remained elevated during the whole analysis period after CHX application.

The results obtained after the application of the CHX gel containing aspartame are shown in Table 2. CHX was effective on reducing MS in saliva, with a statistically significant difference between the baseline values (1.0×10^6 CFU mL^{-1} of saliva) and values observed in all sample collection periods after the CHX application (Wilcoxon test, $p<.05$). Considering that children with an MS level e" 1.0×10^5 CFU mL^{-1} of saliva are classified as high caries risk[4-5], our study compared these MS levels with those observed after the application of CHX gel containing aspartame (Table 2); after 60 days, there was no statistically significant difference was found between the MS levels in this period and the high caries risk MS level.

Table 1: Means and standard deviations of salivary MS levels (CFU mL^{-1} of saliva) before (baseline) and 7 and 30 days after application of the CHX gel containing saccharin (n = 9 subjects).

	Periods of collection of saliva samples		
	Baseline	7 days after CHX application	30 days after CHX application
Mean	1.0×10^6	8.9×10^5	1.0×10^6
s.d.	0	2.2×10^5	0

No statistically significant difference compared to baseline (p>.05).

Discussion

Although epidemiological studies have reported higher caries prevalence and poorer oral hygiene in deaf subjects than in their normal counterparts[22-23], little attention has been paid to caries preventive measures for those patients. This is why the present evaluated the effect of CHX in special care children highly infected with MS, classified as high caries risk patients[4-5]. Our study have shown that CHX gel containing aspartame as sweetener was effective in reducing MS in saliva, being thus important to control the cariogenic activity in deaf children due to their cooperative behavior. However, it is important to highlight that other established evidence-based prevention methods should not be excluded for those children, such as diet modifications and good oral hygiene practices, since dental caries is a disease with a multifactorial etiology.

CHX gel containing saccharin as sweetener was not effective on the reduction of MS in saliva. Similar results were found by Rocha et al. (2003)[25] after application of the CHX gel containing saccharin in volunteers using removable dentures. These findings may be explained by the results obtained *in vitro* by Cury et al. (2000)[17], who demonstrated that the antibacterial activity of CHX may be reduced depending on the concentration of saccharin used to make its taste acceptable. For CHX gel formulations, 1% saccharin is commonly added to make the gel acceptable, especially for children. However, Cury et al. (2000)[17] observed that at concentrations above 0.5% saccharin significantly reduced the anti-mutans activity of 1% CHX gel. Therefore, when saccharin is used, the detrimental effect of concentration on CHX activity should be considered or another non-complex sweetener should be used, such as aspartame.

The aspartame, added to improve the taste of the CHX gel, became it acceptable by the deaf children, without inhibiting the antibacterial activity of 1% CHX gel, since there was a reduction in the children salivary MS after the gel application. Although MS had been decreased to low or undetectable levels by the gel, as observed 7 days after CHX application, the treatment was not able to completely eliminate these bacteria from the mouth. This suggests that there must be reservoirs or retention sites in the dentition hardly affected or not affected at all by chemotherapy, and from which the MS recolonize tooth surfaces after the antimicrobial pressure is removed[6,20]. In this study, CHX treatment was followed by a gradual reappearance of the organisms, where saliva samples obtained after the gel application has shown that MS levels increased with time.

Table 2: Means and standard deviations of salivary MS levels (CFU mL^{-1} of saliva) before (baseline) and 7, 30, 60, 90 and 120 days after application of the CHX gel containing aspartame (n = 9 subjects).

	Periods of collection of saliva samples					
	Baseline	After 7 days	After 30 days	After 60 days	After 90 days	After 120 days
Mean	1.0×10^6	6.7×10^3*†	2.6×10^4*†	8.6×10^4*	1.6×10^5*	4.2×10^5*
s.d.	0	1.7×10^4	3.4×10^4	9.8×10^4	1.5×10^5	3.6×10^5

**Statistically significant difference compared to baseline (p<.05).*
† No statistically significant difference compared to the high caries risk MS level (1.0×10^5 CFU mL^{-1} of saliva) (p.05).

However, even 120 days after CHX treatment the MS levels were significantly different from the baseline values. These results corroborate those of previous studies[6,13,18,20-21] which observed a gradual MS re-appearance during the period of 180 days, after these bacteria had been suppressed to undetectable levels by the treatment with CHX.

Although MS levels was significantly different from the baseline values during the whole evaluation period, a new CHX treatment may be necessary 60 days after the application of the CHX gel containing aspartame, since MS levels observed in this period did not differ significantly from caries risk MS level (e"1.0 x 10^5 CFU mL^{-1} of saliva)[4-5]. This short reapplication period reinforces the opinion that other studies are necessary to improve and develop techniques that promote more lasting reduction periods for deaf children, therefore enabling a better caries-preventive effect of the CHX gel. Similar results were recorded by previous studies with non-handicapped subjects, which found that CHX applications must be repeated within 60 to 90 days[6]. However, the findings of the present study should be interpreted with care, since there was a large intra-individual variation in the time of MS recolonization among the children. Therefore, the effect of CHX treatment must be monitored, given sharp individual variability in response to this treatment.

This large individual variation in response to treatment may be due several reasons, and explain the high standard deviation values found in the present study. Emilson et al. (1987)[21] and Wallman (1998)[27] verified that highly MS-infected dental faces before the CHX therapy were more quickly recolonized, even when MS was suppressed to undetectable levels. This is in agreement with the findings of studies showing that teeth of individuals with several fillings were more rapidly recolonized with MS after CHX than those of subjects with few fillings[18,28-29]. Barkvoll et al. (1989)[30] verified that polysaccharides synthesized from sucrose interfere with the CHX effect, inhibiting its antibacterial activity, suggesting restriction of sucrose consumption during treatment with CHX, for more longstanding effects.

Within the limitations of this study, it can be concluded that CHX gel containing saccharin was not effective in reduction MS levels, while the gel containing aspartame decreased significantly the MS levels in deaf children during the whole evaluation period. Although a new CHX application may be necessary after 60 days to control caries risk MS level, CHX treatment should be individually controlled because of variations in response of the subjects.

Acknowledgements

The authors thank PIBIC/CNPq for the scholarships granted for the first author.

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