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The influence of mouthrinses with antimicrobial solutions on the inhibition of dental plaque and on the levels of *mutans* streptococci in children

Influência de bochechos com soluções antimicrobianas na inibição da placa dentária e nos níveis de estreptococos mutans *em crianças*

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ABSTRACT: The effect of daily mouthrinses on dental plaque accumulation and on salivary *mutans* streptococci was investigated in 200 children. The utilized solutions were: a placebo solution composed of mentholated deionized water (group I); 0.12% chlorhexidine gluconate associated to 0.05% sodium fluoride (group II); 0.2% chlorhexidine digluconate (group III), and 0.5% stevioside mixed with 0.05% sodium fluoride, with pH 3.4 (group IV). In order to verify the effect on plaque formation, the accumulation of plaque was assessed by means of the Löe[12] index, at the beginning and at the end of the experiment, whereas the quantification of cariogenic streptococci was accomplished on three saliva samples collected at 3 different moments: before the first mouthrinse, 24 hours after the first mouthrinse and 1 week after the last mouthrinse. The mouthrinsing routine was carried out on a daily basis during 4 weeks. Five milliliters of solution were rinsed during 1 minute. The results revealed 4.10, 26.75, 41.20, and 5.91% of reduction in plaque accumulation for groups I, II, III, and IV, respectively. Comparisons between the groups as to plaque reduction revealed that groups II and III were significantly different from groups I (control) and IV ($p < 0.05$), but did not differ from each other. The solution utilized by group III was the least accepted by children and, as the solution utilized by group II, caused mild dental pigmentation. There were no statistically significant differences as to the levels of *mutans* streptococci, probably due to the low initial levels observed in each one of the four groups.
UNITERMS: Dental plaque; Chlorhexidine; Sodium fluoride; *Streptococcus mutans*.

RESUMO: Avaliou-se, num total de 200 crianças, o efeito de bochechos diários com solução placebo de água deionizada mentolada (grupo I); gluconato de clorexidina 0,12% associado ao fluoreto de sódio 0,05% (grupo II); digluconato de clorexidina 0,2% (grupo III) e esteviosídeo 0,5% associado ao fluoreto de sódio 0,05% pH 3,4 (grupo IV), sobre o acúmulo de placa dentária e o nível de estreptococos *mutans* salivares. Utilizando-se o índice de Löe[12], o acúmulo de placa dentária foi avaliado no início e no final do experimento, enquanto os níveis de estreptococos *mutans* foram quantificados com o Caritest SM em 3 momentos: antes do primeiro bochecho, 24 horas após o primeiro bochecho e 1 semana após o último bochecho. O regime de bochechos foi diário, com 5 ml de solução, por 1 minuto, durante 4 semanas. Os resultados mostraram uma inibição do acúmulo de placa de 4,10%, 26,75%, 41,20% e 5,91% para os grupos I, II, III e IV, respectivamente. A comparação entre os grupos quanto a redução da placa revelou que os grupos II e III diferiram significantemente dos grupos controle (I) e IV ($p < 0,05$), mas não entre si. A solução do grupo III foi a de menor aceitação pelas crianças e, juntamente com a solução do grupo II, promoveu pigmentações dentárias suaves. Não se registrou diferença significante nos níveis de estreptococos *mutans* em nenhum grupo, provavelmente devido aos baixos valores iniciais observados em todos os grupos.
UNITERMOS: Placa dentária; Clorexidina; Fluoreto de sódio; *Streptococcus mutans*.

INTRODUCTION

Dental plaque formation is a progressive and dynamic process which may result in the establishment of caries and gingivitis. Bacteria are an essential component of plaque and the association between the levels of *mutans* streptococci, as well as of lactobacilli, and the presence of caries in humans is well established[3]. Thus, in order to prevent mouth diseases, plaque control by means of regular mechanical removal, joined or not by agents

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which act specifically against those microorganisms, is fundamental.

Chlorhexidine is considered a safe and effective antiseptic for the reduction of plaque, gingivitis and *mutans* streptococci levels, both in plaque and saliva[16]. Clinical[13,18] and *in vitro*[13] studies have demonstrated that its association with fluoride is particularly effective against *mutans* streptococci. Stevioside is another substance that has also shown anticariogenic[9] and antiplaque[2,17,21] effects, and the evaluation of its association to sodium fluoride in an acid pH is of peculiar interest, since a study has demonstrated that sodium fluoride in acid pH is effective in inhibiting dental plaque accumulation and bacterial metabolism[21].

Considering the importance of dental caries prevention in pediatric dentistry, the restricted ability of children in performing mechanical cleansing, and the necessity of expanding the clinical research on substances that present antiplaque effect, we have carried out this study in order to determine the effectiveness, acceptance and possible side effects of the aforementioned agents.

MATERIALS AND METHODS

Two hundred children aged from 7 to 11 years old, presenting good oral conditions, were selected at the private school Padre Paulo Petruzellis and at the public school Santa Maria, both in Bauru, São Paulo, Brazil, to take part in this study. Firstly, for the evaluation of dental plaque, the initial plaque index (IPI) was determined as proposed by Löe[12], under artificial lighting, using sterilized mirrors and probes. Afterwards, the children were divided into four groups, according to their IPIs, so that the effects of different mouthrinses could be assessed: group I (mentholated deionized water); group II (0.12% chlorhexidine gluconate associated to 0.05% sodium fluoride - Duplak*); group III (0.2% chlorhexidine digluconate - diluted from 10% Plak-Out*); and group IV (0.5% stevioside associated to 0.05% sodium fluoride pH 3.4 - Steviafarma Industrial S/A). Immediately after professional prophylaxis, the children started their first mouthrinse with the specific solution from each group, following a daily routine of mouthrinsing with 5 ml of the solution[21], during 1 minute, for 4 weeks. Mouthrinsing was carried out under supervision during the week days and the children were instructed not to ingest anything for the following 30 minutes. During weekends and holidays the children received a bottle with 5 ml of the solution for each day, as well as usage instructions, which were also given to their parents. On the day after the last mouthrinse, the final plaque index (FPI) was registered and an oral examination was carried out to detect the presence of pigmentation on teeth, tongue and oral mucosa, or any other side effects. On the last week of the experimental phase, the children answered a survey which supplied us with information on the acceptance of the utilized solutions.

The effects of the solutions on the levels of *mutans* streptococci were evaluated on stimulated saliva samples collected from 20 children of each group, which were randomly selected. Following the instructions of the manufacturer of Caritest SM (HERPO - Produtos Odontológicos Ltda) the quantification of microorganisms was carried out on three samples of saliva, which were collected at the beginning of the study (before professional prophylaxis), 24 hours after the first mouthrinse, and 1 week after the last mouthrinse. During the assessment, criteria such as a standardized period of sample agitation (30 seconds), volume of saliva added to the buffer solution (1.5 ml) and culture examination by means of a stereoscopic microscope – to verify the morphology of colonies – were observed. Basically, on *mitis salivarius* bacitracin two types of colonies were found. They corresponded to *S. mutans* and *S. Sobrinus*. According to Emilson[4] (1983), the evaluation of colony morphology is a convenient means to identify *mutans* streptococci.

In the analysis of the results, both mean and percentual values were utilized. The Kruskal-Wallis analysis of variance was employed for comparisons between all groups. Individual comparisons between groups were carried out with Dunn's test, and comparisons within the groups, with Friedman's test. The significance level was 5%.

RESULTS

The results registered on Table 1 are the percentages of IPI and FPI related to scores 0, 1, 2, and 3 of the Löe[12] (1967) index. Table 2 shows the average and standard deviation of initial and final plaque indexes in each group, as well as the percentage of plaque reduction. To compare the groups as to the reduction of plaque index, a model of Kruskal-Wallis analysis of variance was utilized

*Dentsply - HERPO.

TABLE 1 - IPI and FPI percentual values in the four studied groups.

Groups	I		II		III		IV	
Score	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	5.89	10.44	5.58	21.98	6.05	35.92	6.39	9.33
1	38.75	34.57	37.01	45.57	39.35	41.92	35.96	38.63
2	53.23	54.28	52.72	31.24	52.72	21.78	56.89	51.96
3	2.13	0.71	1.82	1.21	1.88	0.38	0.76	0.08
	100	100	100	100	100	100	100	100

IPI = initial plaque index; FPI = final plaque index.

TABLE 2 - Average (X) and standard deviation (sd) of IPI and FPI. Percentual reduction of the plaque index in the studied groups.

Groups	IPI (X ± sd)	FPI (X ± sd)	Diference (X ± sd)	Reduction (%)
I	1.513 ± 0.292	1.451 ± 0.294	0.062 ± 0.217	4.10
II	1.532 ± 0.311	1.122 ± 0.325	0.410 ± 0.285	26.75
III	1.502 ± 0.309	0.866 ± 0.383	0.637 ± 0.404	41.20
IV	1.522 ± 0.264	1.431 ± 0.294	0.091 ± 0.164	5.91

IPI = initial plaque index; FPI = final plaque index.

and revealed statistically significant differences ($p < 0.05$). During individual comparisons by means of Dunn's test (Table 3), groups II and III demonstrated statistically significant differences when compared to groups I (control) and IV.

Based on the data obtained in this study, it was observed that, in groups presenting with significant reductions, 65.96% (group II) and 20.93% (group III) of the children classified the taste of the utilized solution as "pleasing". The percentages of children who reported unpleasant taste in groups I and IV were 12.82% and 13.89%, respectively. Mild pigmentation was observed in 14.89% of the participants from group II, and in 27.91% of the children from group III. No pigmentation occurred in group I and IV.

The number of children related to each one of the levels of *mutans* streptococci, in each group, is depicted in Table 4. Comparisons between the three levels of *mutans* streptococci conunts, carried out by means of Friedman's test, did not reveal statistically significant differences between the groups ($p < 0.05$).

DISCUSSION

Table 1 highlights the effectiveness of the solutions utilized by groups II and III, which is revealed by the increase in the prevalence of score 0 and by the decrease in the prevalence of scores 2 and 3 in the final evaluation. That confirms the antiplaque effect of these solutions, as well as their value for clinical application on specific situations that might require better plaque control in children. Such an effectiveness proves relevant in the comparisons shown on Tables 2 and 3.

TABLE 3 - Results of Dunn's test for individual comparisons between the groups as to plaque reduction.

Groups	Difference	Critical values
I x II	53.10*	29.004
I x III	78.09*	28.563
I x IV	3.01	29.499
II x III	24.99	28.050
II x IV	50.09*	29.004
III x IV	75.08*	28.563

*Significant ($p < 0.05$).

The effect of chlorhexidine associated to sodium fluoride against plaque and dental caries has been previously proven in other studies[8,18]. In addition, it has already been demonstrated that mouthrinses with chlorhexidine, in different concentrations, frequencies of use, and quantities, prevent the formation of dental plaque[11,15]. Employing the same rou-

TABLE 4 - Number of children per group and salivary *mutans* streptococci (MS) levels in three different occasions*.

Groups	I			II			III			IV		
MS levels (UFC/ml)	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
UD**	3	1	2	3	2	5	0	3	0	7	5	5
< 100.000	11	16	13	11	14	13	14	14	15	9	9	8
250.000	2	1	1	0	1	0	4	2	2	2	5	4
500.000	2	0	1	4	1	0	0	0	1	2	1	2
1.000.000	0	0	1	0	0	0	1	0	1	0	0	1
Total	18			18			19			20		

*Before (1st), 24 hours after the first mouthrinse (2nd) and one week after the last mouthrinse (3rd); **undetectable.

tine as that carried out in group II during 6 weeks, Zanela[21] (1995) observed a reduction of 52.63% in the mean score of plaque, which is in agreement with the findings of the present study.

The reduction in the accumulation of dental plaque was not evident in group IV (Table 2), even though clinical studies using mouthrinses with stevioside in children[2,21], as well as in adults[17], have evidenced statistically significant reductions in the accumulation of dental plaque, which varied from 23% to 50%. Such inconsistency might be due to the higher frequency of use (4 times a day) and to the concentration (10%) employed[17]. In the study carried out by Zanela[21] (1995), the concentration of stevioside was lower (0.1%), no fluoride was added to the soloution, and the pH was neutral. Even so, the percentage of plaque reduction was 30.6%. However, it cannot be concluded that stevioside was inactivated by the pH of the solution in the present investigation, since this substance is stable in a wide pH range[7]. Additionally, in the concentration employed in group IV (0.5%), stevioside had previously demonstrated antiplaque[2,7] and anticariogenic effect[7]. Another possible explanation for the poor performance of the association between stevioside and sodium fluoride is the low solubility of the 0.5% solution at room temperature, which alters its concentration and may be accentuated after a period of time.

Regarding the acceptance of mouthrinses by children, except for group III, the predominant opinion was positive. According to the children, the solution used in group III has a somewhat strong taste – even an unpleasant taste for some of them – as it was also observed by Zanela[21] (1995). Side effects have been reported after the utilization of chlorhexidine, specially regarding its bitter taste. The presence of dental pigmentation in groups II and III confirms this side effect as the most frequently detected in the majority of the studies on chlorhexidine, in adults and children[11,21]. Disregarding the alteration observed in these two groups, no irritation or desquamation of the oral mucosa were observed, neither were burning or dryness symptoms reported.

The literature has shown that the substances tested in this study act on *mutans* streptococci[1,18,19,22]. However, during this research, there were no statistically significant differences between the levels of these microorganisms in the saliva, in the three evaluation periods, for any of the three modes of treatment.

In groups II and III, but not in group IV, the solutions produced a very good effect only on those children who presented with high levels of *mutans* streptococci at the beginning of the study. The other children registered low levels – in all groups, about 20% of the children showed levels of *mutans* streptococci ≥ 250.000 – which probably contributed to the apparent lack of antimicrobial potential. This confirms that chlorhexidine is specifically indicated to suppress *mutans* streptococci in oral cavities colonized by high levels of such microorganisms[5]. It should be noted that children who presented a decrease in bacterial levels did not present erupting permanent teeth – those are important reservoirs that contribute to relatively high scores of *mutans* streptococci, as it occurs during the eruption of third molars[14]. It was observed that the recomended daily dosage of the most efficient method is higher than the one employed in group III, which was of 10 mg/day (5 ml once a day), since it was adapted to children of this age group[2,21].

The combination of chlorhexidine and fluoride has an effect against plaque, accumulation of cariogenic microorganisms and caries[5]. According to Emilson[5] (1994), the small fluoride ion can reach *mutans* streptococci which survive in retention sites and in incipient enamel lesions, interfering with their metabolic activities and, thus, contributing to delay their reappearance. Another possibility, according to Spets-Happonen et al[18] (1991), is the decrease of the ecological advantage of these bacteria in retention sites with low pH, since acid production is inhibited to a greater extent by the combination of chlorhexidine and fluoride. Twetman, Peterson[19] (1997), employing varnish, also demonstrated a better effect on *mutans* streptococci when the substances were combined, emphasizing that, despite the utilized method, a probable synergic action can occur.

Although the population under investigation had attended a previous preventive program, the clinical conditions presented were heterogeneous, and the caries risk was uncertain. The assessment of caries risk is important for pediatric dentistry in order to concentrate preventive procedures on high-risk patients. However, it is difficult to establish that risk since it involves the survey of many elements such as previous caries history, plaque index, counts of cariogenic microorganisms, diet, occlusal morphology (pits and fissures), presence of incipient active caries in smooth surfaces and the psychological, physical, social and cultural history of the individual. The level of *mutans* streptococci allows for the identification of high-risk patients and monitors the established treatment whenever there is suspicion of high risk[10]. The evaluation is applicable to young children, even before the development of any carious lesion[20], since the colonization by these bacteria precedes the development of clinically detectable carious lesions[6].

This study confirms the antiplaque effect of solutions containing chlorhexidine. It also confirms the importance of *mutans* streptococci levels in detecting children at high risk for dental caries, who are potential candidates to the utilization of those solutions. On the other hand, the results point out the need to reassess preventive programs in order to restrict the utilization of antimicrobial solutions, whose indication must be based on distinct criteria and on the multifatorial nature of dental caries.

CONCLUDING REMARKS

Chlorhexidine solutions, associated or not to fluoride, significantly reduce dental plaque formation, but do not reduce the levels of *mutans* streptococci unless they are initially high, in groups with the conditions presented in this study. Both solutions produced mild dental pigmentation and alterations in taste for some of the children, although no other side effects were observed; therefore, they can be recommended by the pediatric dentist as aids in plaque reduction, in specific situations. The association of 0.5% stevioside and 0.05% sodium fluoride did not present satisfactory results. However, due to other promising results, it deserves new investigations.

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