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Sodium fluoride and chlorhexidine effect in the inhibition of mutans streptococci in children with dental caries: a randomized, double-blind clinical trial

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Objective: We aimed to compare the effect of sodium fluoride and chlorhexidine on salivary levels of mutans streptococci (MS), in a double-blind, randomized clinical trial.

Methods: Thirty-five healthy volunteers, aged 4–8 years, with at least one active carious lesion and no previous history of allergies were selected to participate in the study. A gel formulation containing either 1.23% sodium fluoride or 1% chlorhexidine was topically administered to the dentition every 24 h for 6 consecutive days. Salivary MS levels were measured at baseline (D1) and on the 6th (D6), 15th (D15), and 30th (D30) days. For microbiological analysis, Mitis Salivarius-Bacitracin agar medium was used.

Results: Difference between treatments was only verified on D6. On the last day of treatment 1% chlorhexidine gel was significantly more effective than fluoride ($P = 0.0000$). The use of sodium fluoride did not cause a statistically significant variation in salivary MS levels throughout the duration of the study. Following treatment, a subsequent increase in MS counts between D6 and D15 ($P = 0.0001$) was observed with chlorhexidine.

Conclusion: A 6-day treatment with a 1% chlorhexidine gel was effective in reducing salivary MS; there was a significant MS increase once treatment was suspended. The use of 1.23% sodium fluoride under the same regimen was not able to reduce salivary MS levels. Our results suggest repeated treatment with 1% chlorhexidine as a means for maintaining low salivary MS levels in children with dental caries.

Key words: chlorhexidine; dental caries; fluoride; mutans streptococci

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Dental caries is an infectious disease of bacterial origin that could benefit from the use of topically applied antimicrobial agents generating a favorable ecological shift within the oral environment. Mutans streptococci (MS), a phenotypically similar group of bacteria, have been identified as the main pathogens involved in dental caries development. Studies have verified that maternal salivary levels of MS are related to dental caries in the offspring (3). While salivary contamination with these

microorganisms has a direct correlation with caries risk in children, adolescents, and adults, oral suppression of MS can significantly reduce the risk of developing this disease (24). These pathogenic bacteria, along with other members of the indigenous biota, share the ability to aggregate and form a microbial biofilm on dental surfaces. Since plaque-associated diseases tend to remain localized rather than becoming invasive, topically applied rather than systemically administered antimicrobial therapies may be more effective, with the advantage that the patients are submitted to short exposure times.

The use of antimicrobial agents in the treatment of this infection has been studied for over five decades (2, 9). Fluoride has been indicated as a major contributor in the treatment and prevention of dental caries because of its cariostatic and remineralizing properties. Previous work has demonstrated *in vitro* antibacterial effects against MS (6, 8); however, the clinical antimicrobial value of these formulations has been questioned. While an observed caries reduction with the use of topical fluoride is frequently a fact (25), decrease in the prevalence of this disease has not always been associated with a decline in the numbers of MS (11, 33), leading to controversies as to its antibacterial properties. Furthermore, the clinical use of fluoride preparations for antimicrobial purposes has had little acceptance among dental practitioners because the administration of fluoride preparations requires large concentrations and a high frequency of application, surpassing the concentration needed to reduce enamel demineralization (34).

Chlorhexidine is a broad-spectrum antimicrobial agent, with activity against streptococci, actinomyces, gram-negative rods, yeasts, total aerobes, and total anaerobes. High concentrations of chlorhexidine have an immediate bactericidal effect, penetrating the bacterial cell wall and leading to precipitation of the cytoplasm (19), whereas lower concentrations are bacteriostatic. Clinical application of chlorhexidine within the oral cavity, in the form of gel or varnishes, has proved effective in MS reduction in both saliva and dental plaque (29). An unpleasant taste, tooth staining after long-term use, and the need for frequent applications have stimulated the search for new and more appropriate alternatives for use in young children (1). Therefore, although considered as the gold standard in the inhibition of bacterial plaque formation (27), the antimicrobial application of chlorhexidine for dental caries has been questioned (35). Nevertheless, other agents lack its favorable chemical properties because a great advantage of chlorhexidine use over other antimicrobial substances lies in its prominent substantivity – chlorhexidine is retained in the oral surface generating a slow release and longer lasting effect – coupled with the lack of significant side effects and bacterial resistance, being well tolerated by pediatric patients (12, 27).

The aim of the present study was to compare salivary MS levels in two different groups of children with caries that had received intraoral topical treatment with either 1.23% sodium fluoride or 1% chlorhexidine for 6 consecutive days. This clinical trial was designed to test two different hypotheses. First, that sodium fluoride alone would demonstrate effectiveness in salivary MS reduction in children with dental caries. Second, a 6-day antimicrobial treatment with either one of these agents would produce a long-lasting MS suppression, dispensing repeated antibacterial treatments.

Materials and methods

Population

The study protocol was approved by the Ethics Committee of the Federal University of Ceará Medical School, Brazil and complies with the current Brazilian laws. After written informed consent was signed by parents or legal guardians, 35 volunteers, aged 4–8 years, from both genders, with at least one carious cavitated or non-cavitated lesion, were recruited to participate in the study from a population of 400 children searching for dental care at the Pediatric Dental Clinic of the Federal University of Ceará (Table 1) by one graduate and one postgraduate student from the same university. Children with a history of allergies or allergic diseases, e.g. asthma, urticaria, rhinitis, sinusitis, or intraoral soft tissue lesions, were excluded from the study. None of the participants underwent antibiotic treatment during the course of this clinical trial.

Treatment application

Patients were randomly assigned to either one of two different groups, by using a lottery system. Each group received topical treatment with either a 1.23% sodium fluoride (from PA solution, distributed by Cinética, Fortaleza, Brazil), or 1% chlorhexidine digluconate (from 20% stock, distributed by Sinty, Fortaleza, Brazil). Treatments were formulated as gels with similar color and taste by the Laboratory of Pharmaceutical Science at the Federal University of Ceará, Fortaleza, Brazil (Table 2). The identification of each substance used was concealed from the postgraduate student in charge of applying the treatment, and from the study participants, until the clinical trial was concluded. Once patients enrolled to participate in the clinical trial, an identification number was sequentially assigned, allowing maintenance of a double-blind, randomized study design.

Before the start of treatment, a clinical examination was performed by only one examiner, using a visual/tactile method to calculate the number of decayed, missing,

Table 1. Number of carious surfaces, decayed, missing and filled surfaces (dmfs) scores, age and gender distribution, expressed in mean ± SD, for each treatment group

					Gender	
Treatment groups	*n*	Age (months)	dmfs	Carious surfaces	Females	Males
NaF	17	75 ± 16.6	8 ± 4.7	7 ± 4.7	10	17
CHX	18	78 ± 18.9	10 ± 7.4	8 ± 7.0	6	12
Total	35				16	19

NaF, sodium fluoride; CHX, chlorhexidine.

Table 2. Gel formulations used for topical treatment in the two different study groups

Constituents	NaF	CHX
Sodium fluoride (g)	1.23	–
Chlorhexidine digluconate 20% (ml)	–	5.00
Methylparaben (g)	0.135	0.135
Propylparaben (g)	0.015	0.015
Hydroxyethylcellulose QP 250 (g)	1.15	1.15
Steviosideo (g)	0.24	0.24
Glycerol (g)	5.00	5.00
Water q.s.p. (g)	100.00	100.00

NaF and CHX corresponds to sodium fluoride and 1% chlorhexidine, respectively. Amounts of all constituents are expressed in g/volume, with the exception of chlorhexidine, which is expressed in ml/volume.

and filled surfaces, and further follow-up of these patients was arranged. Subsequently, alginate impressions and plaster models were obtained for custom tray fabrication, to assure treatment application to the dentition only, avoiding the soft tissues and minimizing systemic absorption. Each treatment gel was placed inside the trays, as a 3-ml volume, and applied for 10 min, every 24 h, for 6 consecutive days.

Saliva collection and microbiological analysis

Saliva was collected before treatment initiation (D1), on the last day of treatment (D6), 15 days after day 1 (D15) and 30 days after day 1 (D30). Patients were asked to chew on a 3 × 3 cm piece of Parafilm® for 60 s to stimulate saliva secretion and release plaque into the salivary fluid. Saliva was then collected with a disposable plastic cannula and stored in sterile Eppendorf tubes for subsequent analysis. Samples were transported to the laboratory for microbiological analysis in a hermetically sealed case containing ice, and analyzed no longer than 2 h after collection (31).

A 0.1-ml volume was aseptically drawn from each sample and transferred into one sterile test tube containing 0.9 ml saline. This procedure was repeated twice, establishing dilutions of 1 : 10 and 1 : 100. A corresponding volume of 10 µl of each dilution was plated onto Mitis Salivarius-Bacitracin (MSB) agar medium (18) in triplicates. The plates were then incubated at 37°C for 48 h, in jars under microaerophilic conditions. Representative colonies with morphological characteristics of MS were counted, isolated, and biochemically confirmed to be MS utilizing mannitol, sorbitol, lactose, raffinose, melibiose, and esculin. Bacterial counts were expressed as colony-forming units (CFU)/ml of saliva.

Statistical analysis

Data are presented as median, lower to upper quartiles, and mean values ± standard deviation (SD). The results of comparisons were considered statistically significant when $P < 0.05$. Comparisons of number of CFUs between groups and between the different days of treatment were performed by applying Mann–Whitney and Wilcoxon tests. Spearmann's correlation coefficient was used to verify the correlation between number of carious surfaces and salivary MS concentrations.

Results

No important side effects were described by children or parents, other than two reports of tooth staining; this was easily removed by dental prophylaxis once the clinical trial was concluded.

Comparisons between and within treatments on different days

A difference in salivary MS levels between treatments was only verified on D6. Chlorhexidine treatment demonstrated a significantly higher efficacy in MS reduction when compared to sodium fluoride (Mann–Whitney test, $Z = 4.92$, $P = 0.0000$). The use of sodium fluoride did not cause a statistically significant variation in salivary MS levels throughout the duration of the study. No MS reduction was observed when comparing MS levels at D1 and D6, in the sodium fluoride group ($P = 0.6192$) (Tables 3 and 4). A statistically significant MS reduction between D1 and D6 was observed with 1% chlorhexidine gel ($P = 0.0001$); however, a significant increase in MS levels between D6 and D15 was noted with this treatment ($P = 0.0001$) (Fig. 1).

Correlation between number of carious surfaces and MS counts

In the group treated with fluoride, the number of carious surfaces expressed a positive correlation with contamination levels in D15 ($P = 0.0001$), D30 ($P = 0.0421$), and with the MS increase observed between D6 and D15 ($P = 0.0143$). In contrast, the group of children treated with chlorhexidine showed a statistically significant negative correlation between the number of carious surfaces and the bacterial rise observed between D15 and D30 ($P = 0.0438$) (Table 5).

Discussion

It is fully understood that success of antimicrobial treatment against dental caries, regardless of the substance applied, should be measured considering to its impact on caries as the final outcome (7). Nevertheless, an initial effect on MS salivary levels would be an adjunct for the reduction of cross-contamination between mother and child (23), clinical treatment of lesions and subsequent reduction on recurrence of dental caries, if appropriately associated with other means for prevention. While the use of chlorhexidine as an antiplaque and antigingivitis agent remains a gold standard (20), its use as an anticaries agent has been considered as controversial, in the light of inconclusive clinical evidence (35). Chlorhexidine results in the present study confirmed those from previous studies reporting that it strongly reduces MS (13, 16) but it still allows recolonization (14), especially when high levels of MS are detected before treatment (15). When observing the number of carious surfaces and the rise in bacterial levels, chlorhexidine has a

Table 3. Salivary mutans streptococci levels of patients who received a 6-day topical gel treatment with either 1.23% sodium fluoride (NaF) or 1% chlorhexidine (CHX)

Day	Treatment	*n*	Mean	SD	Minimum	Median	Maximum
D1	NaF	17	42,620	131,290	30	10,330	550,000
	CHX	19	13,860	11,530	730	13,380	35,550
D6	NaF	17	14,960	19,970	200	5870	72,050
	CHX	19	240	720	0	0	3030
D15	NaF	17	26,560	44,610	180	9720	163,670
	CHX	19	15,300	19,440	70	4450	55,000
D30	NaF	17	32,500	42,590	470	17,100	152,330
	CHX	19	14,150	12,980	1070	9500	53,330

Number of subjects (*n*) in each study group, median and mean ± SD of the number of colony-forming units (CFU)/ml are expressed at baseline (D1), and on the 6th (D6), 15th (D15) and 30th (D30) days after D1. Lower to upper quartiles are given.

Table 4. Wilcoxon test for comparison of mutans streptococci levels between days of treatment, for the different treatments analyzed

		Days			
Treatments	Specifications	D1 vs. D6	D6 vs. D15	D15 vs. D30	D1 vs. D30
Sodium fluoride	*Z* statistics	0.497	0.970	0.923	1.728
	Significance	0.6192	0.3318	0.3560	0.0840
Chlorhexidine	*Z* statistics	3.825	3.823	0.443	0.121
	Significance	0.0001	0.0001	0.6580	0.9039

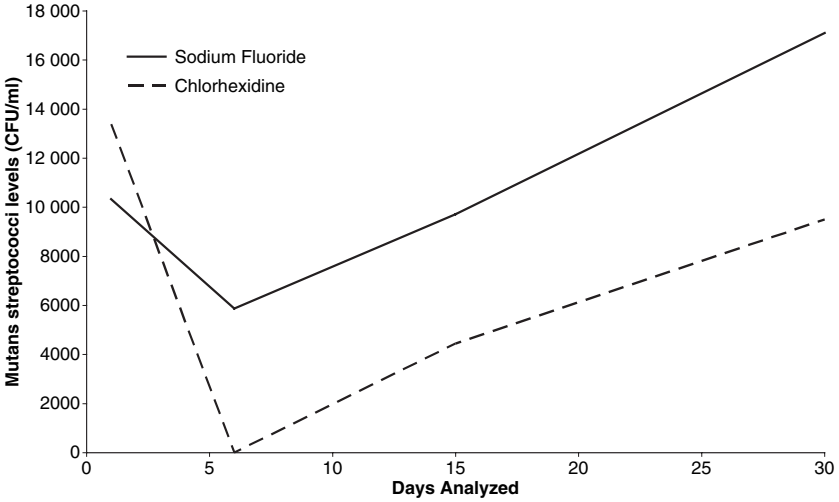

Fig. 1. Comparisons between median values of salivary mutans streptococci levels [colony-forming units (CFU)/ml] for sodium fluoride and chlorhexidine, from day 1 (before treatment application) through to day 30.

Table 5. Correlation between number of carious surfaces and contamination levels with mutans streptococci on the various days of treatment (Spearman correlation)

	D1[1]	D6	D15	D30	Red.[2] 1–6	Inc.[3] 6–15	Inc. 15–30	Inc. 1–30
Sodium fluoride treatment								
Correlation coefficient	0.262	0.397	0.811	0.498	−0.131	0.582	−0.063	0.394
Significance	0.3090	0.1143	0.0001	0.0421	0.6157	0.0143	0.8098	0.1180
n	17	17	17	17	17	17	17	17
Chlorhexidine treatment								
Correlation coefficient	0.072	−0.226	0.443	−0.087	0.076	0.443	−0.480	0.012
Significance	0.7770	0.3668	0.0659	0.7306	0.7632	0.0659	0.0438	0.9608
n	18	18	18	18	18	18	18	18

[1]Baseline (D1), on the 6th (D6), 15th (D15) and 30th (D30) days after D1.
[2]Reduction (Red.) in mutans streptococci levels between D1 and D6.
[3]Increase (Inc.) in mutans streptococci levels between D6 and D15; D15 and D30 and D1 and D30.

tendency towards a negative correlation between these variables, possibly because of its favorable chemical properties. In addition to binding to the negatively charged bacterial cell wall, exerting a bacteriostatic or bactericidal effect, chlorhexidine binds to the oral surfaces, dental pellicle, and saliva (30). Hence, the presence of more carious surfaces would favor its retention and subsequent release, possibly resulting in a longer lasting antimicrobial effect, responsible for a lower increase in salivary MS levels in the presence of a higher number of carious lesions.

The group of children that received topical treatment with 1.23% sodium fluoride for 6 consecutive days did not show any decline in the levels of MS present in the saliva. With the lack of clinical evidence, the use of fluoride as an antibacterial agent remains controversial (36). However, *in vitro* experiments have demonstrated the ability of fluoride to selectively reduce MS in artificial plaque models (4), as well as to exert specific effects on the metabolism and acid tolerance of oral bacteria (26). It has been suggested that future research related to the antibacterial effects of fluoride should be aimed at bridging the gap between experiments *in vitro* and *in vivo* (36). While many studies have been able to report reductions in MS levels following short-term or long-term treatment with fluoride (22, 37), in the past, others have failed to demonstrate such action (28). Hence, although *in vitro* antimicrobial properties of sodium fluoride have been demonstrated (5), the clinical value of such formulations in the carious process might be predominantly a product of its anticaries efficacy (17). In spite of a known fluoride uptake by sound and carious surfaces (32), a positive influence of the number of carious lesions on the bacterial levels observed following treatment with this agent, confirms this hypothesis. The presence of cavitated lesions creates bacterial niches, imposing stronger limitations on the weak or non-existent clinical antibacterial effect of this substance, and so favoring the observed MS increase.

Regardless of the substance tested, MS suppression has been repeatedly and successfully attained; however, once treatment is suspended, MS overgrowth has been a common pattern in clinical trials with both adults and children. In 1977, De Paola et al. (10) performed a clinical trial with an antimicrobial agent, in a population of 268 children, aged 9–11 years. In their study, a follow-up was performed for new carious lesions, while *Streptococcus mutans* populations were monitored in conjunction and reported separately (21). Interestingly, it was noted that the most significant effect in both dental caries control and *S. mutans* reduction was seen in fissure surfaces and

newly erupting teeth. In addition, the most prominent effect in *S. mutans* reduction was observed where higher contamination levels were present. Both clinical and bacteriological data expressed the same pattern. The interruption of treatment application during the holiday period led to an increase in *S. mutans* index, described by the authors as a distinct loss of effect. Treatment continuity would therefore be a determining factor in the final outcome, and the lack of it in our short clinical trial may be another explanation for the MS increase observed in our results with the use of a 1% chlorhexidine gel.

Many attempts have been made to identify an antimicrobial agent, active against MS, for use in children with early childhood caries. In the search for an ideal substance, the same problems encountered earlier with topical antibiotics are currently seen with other agents. Zhan et al. (38) reported on a clinical trial in which 22 children received a one-off treatment with either 10% povidone iodine or phosphate-buffered saline. Reductions in mutans streptococci and lactobacilli were verified for up to 3 months; however, no significant difference in caries increment was found between the two groups, and 60% of subjects had new caries lesions in each group after a 1-year follow-up. Hence, the answer might not be in the substance itself, but in whether long-term MS suppression is a feasible option. If so, the best agent to use should be the one with the narrowest spectrum of activity and the widest therapeutic range. The present study has demonstrated significant MS reduction with a 1% chlorhexidine gel, while 1.23% sodium fluoride lacked the ability to reduce MS in the saliva of children with dental caries. In our study the one treatment capable of producing significant MS reduction, generated a substantial bacterial rise once treatment was suspended, suggesting that continuance of the antimicrobial therapy is the key for maintaining adequate suppression of cariogenic bacteria in populations of children with dental caries who are highly contaminated with MS. Furthermore, the intensive 6-day treatment strategy did not invalidate the need for further antimicrobial therapy. In conclusion, our results suggest that the use of sodium fluoride in children with dental caries should be restricted to its anticaries properties, while chlorhexidine gel, though highly effective in salivary MS reduction, requires repeated applications to maintain long-term salivary MS suppression, showing superior antibacterial efficacy in the presence of a higher number of carious surfaces.

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