Pesqui Odontol Bras
2003;17(1):78-81

Prevenção

Evaluation of the substantivity of chlorhexidine in association with sodium fluoride *in vitro*

Avaliação da substantividade da clorexidina na associação com fluoreto de sódio in vitro

Carolina Saliba de Freitas*
Henrique França Oliveira Diniz*
Jânderson Breder Gomes*
Rubén Dário Sinisterra**
María Esperanza Cortés***

ABSTRACT: The efficacy of the fluoride-chlorhexidine association in the prevention of gingivitis and caries has been advocated for a number of years[5,7,14]. The objective of the association of these therapeutic agents is a synergistic action. The aim of the present study was to determine the substantivity of chlorhexidine associated or not to sodium fluoride at different intervals of time, *in vitro*. Bovine enamel surfaces were treated with 0.12% chlorhexidine gluconate (Periogard® – Colgate®) or 0.05% sodium fluoride with 0.12% chlorhexidine (Duplak® – Dentsply®) solutions for one minute. Fragments were placed in distilled water and samples were collected at intervals of 5, 30 and 360 minutes and analyzed by spectrophotometry in the visible ultraviolet region. Substantivity was evaluated by the measurement of chlorhexidine desorption from the treated slabs. The mean values obtained were statistically analyzed by Student's *t*-test. The results showed that the concentration of chlorhexidine decreased when it was used in association with sodium fluoride. The difference between the groups was statistically significant ($p < 0.05$). These *in vitro* results suggest that the association between sodium fluoride and chlorhexidine in the studied concentrations appears to have no beneficial effect because of the decrease in the substantivity of chlorhexidine.
DESCRIPTORS: Chlorhexidine; Sodium fluoride; Substantivity.

RESUMO: A efetividade da associação fluoreto-clorexidina na prevenção da gengivite e da cárie vem sendo defendida há alguns anos[5,7,14]. O propósito dessa associação é obter um desempenho sinérgico. O objetivo do presente estudo foi avaliar a substantividade da clorexidina em associação ou não ao fluoreto de sódio em diferentes intervalos de tempo, *in vitro*. Foram tratadas superfícies de esmalte bovino com soluções de digluconato de clorexidina 0,12% (Periogard® – Colgate®) ou fluoreto de sódio 0,05% com clorexidina 0,12% (Duplak® – Dentsply®), durante um minuto. A substantividade foi medida pela dessorção da clorexidina a partir dos fragmentos previamente imersos nas soluções teste e colocados em água destilada. Alíquotas foram removidas em intervalos de 5 minutos, 30 minutos e 6 horas e analisadas através de espectroscopia na região do ultravioleta visível. Os valores obtidos foram analisados estatisticamente pelo teste *t* de Student. Os resultados mostraram que a concentração da clorexidina foi significativamente reduzida quando em associação com fluoreto de sódio ($p < 0,05$). Os resultados *in vitro* sugerem que a associação da clorexidina ao fluoreto de sódio nas concentrações estudadas parece não ter efeito benéfico, devido à redução da substantividade da clorexidina.
DESCRITORES: Clorexidina; Fluoreto de sódio; Substantividade.

INTRODUCTION

Chemotherapeutic agents are used as adjuncts to the mechanical control of plaque[15]. In 1890, Miller[10] suggested an appropriate and intelligent use of antiseptic to destroy bacteria, or limit bacterial activity, as a way of inhibiting the destruction caused by dental decay. The main challenge in preventive dentistry continues to be the reduction or control of the acquired film in order to prevent caries and periodontal disease. Several researches have been carried out and the industry continually launches new formulations capable of interfering either in the metabolic activity or the bacterial adhesion to the dental plaque.

Fluoride is the most prominent drug used as an auxiliary in the prevention by enamel remineralization. Fluoride has been used in caries prevention since 1940. Modern concepts of the mecha-

* Undergraduate students, School of Dentistry; **Assistant Professor, Department of Chemistry, Institut of Exact Science;
***Assistant Professor, Department of Restorative Dentistry, School of Dentistry – Federal University of Minas Gerais.

nism of action of fluoride recommend daily fluoride supply to establish and maintain a significant concentration in saliva and in plaque fluids, thus preventing and controlling enamel dissolution[15]. Furthermore, the basis of its application is that after application, either as topical solutions or as toothpaste, the free fluoride is incorporated into the enamel in the form of calcium fluoride on the tooth surface and serves as a fluoride reservoir whenever it is necessary in the demineralization/remineralization process. The use of this agent as chemoprophylaxis or chemotherapeutics in dental decay is still stimulated by the lack of evidence of harmful effects.

Although fluoride acts in the demineralization/remineralization equilibrium, this function is not exercised in pH lower than 4.5, because at this levels the dental plaque is not saturated by fluorapatite. Furthermore, there are no evidences to show the direct benefits of fluoride on individuals with gingivitis alone[17]. At low concentrations, chlorhexidine can reduce plaque acid production. Based on these principles, the fluoride-chlorhexidine association would be quite beneficial[11], probably because chlorhexidine reduces plaque acid formation for several hours, preventing the decrease in pH, and additionally, this drug has great effectiveness in the reduction of gingivitis.

Chlorhexidine, a cationic agent, has been used for more than fifteen years in Europe, and its hydrophobic and hydrophilic properties are responsible for its efficacy. Chlorhexidine is a very potent chemoprophylactic agent. It has a broad-spectrum action, and is more effective against Gram-positive than Gram-negative bacteria. *Streptococcus* of the *mutans* group is especially susceptible to chlorhexidine, which acts by binding to the bacterial cellular wall and affecting its functions[15]. Chlorhexidine binds readily to negatively charged bacterial cell walls and can thereby disrupt membrane integrity. In high concentrations, chlorhexidine is bactericidal and acts as a detergent by damaging cell membrane. One positive property of chlorhexidine is its intrinsic ability to be retained by oral surfaces, and gradually released into oral fluids over many hours, which is defined as substantivity[15].

The fluoride-chlorhexidine association was proposed three decades ago based on results obtained firstly by *in vitro* and later by *in vivo* studies. Luoma *et al.*[7] (1978) and Emilson *et al.*[3] (1976) justified this association as the search for a synergistic effect of the two drugs to control caries and gingivitis. Ullfoss *et al*[15] (1994) reported that a combination of a 2.2 millimolar chlorhexidine mouth rinse used twice a day with single daily rinses of 11.9 millimolar NaF resulted in mineral loss only slightly larger than that observed in sound enamel.

MATERIALS AND METHODS

Bovine teeth were sectioned with a water-cooled diamond saw to obtain the crowns. The 40 fragments obtained were embedded in polystyrene resin and vestibular surfaces of 4 x 8 mm were left exposed. After that, the resin surfaces were covered with nail polish and randomly divided to evaluate the substantivity in Group 1, by one-minute immersion in 0.12% chlorhexidine gluconate solution (Periogard®), or the association of 0.05% fluoride sodium and 0.12% chlorhexidine (Duplak®) in Group 2. The samples were immersed in 1 ml of distilled and sterilized water and kept in glass tubes. After 5, 30 and 360 minutes, an aliquot was taken from the tubes and the same volume was immediately replaced and analyzed by ultraviolet in a spectrophotometer at 260 nm. The values obtained were analyzed with Student's *t*-test.

RESULTS

Graph 1 shows the chlorhexidine release from teeth impregnated with 0.12% chlorhexidine gluconate and those impregnated with 0.05% sodium fluoride and 0.12% chlorhexidine. The peak desorption of chlorhexidine in the first 5 minutes (Table 1) was lower for the sodium fluoride-chlorhexidine association (12.14 μg/ml) than that of the chlorhexidine gluconate solution (16.68 μg/ml). After 30 minutes, there was a sharp fall in the concentration of the two groups as follows, Group 1, 45% (9.16 μg/ml), and Group 2, 82% (2.10 μg/ml). Chlorhexidine concentration corresponding to 360 minutes was 4 μg/ml for chlorhexidine gluconate, whereas sodium fluoride-chlorhexidine had a concentration of only 1.51 μg/ml. However, one of the most important properties of chlorhexidine is its substantivity, which was shown to be reduced in all time intervals analyzed for the association.

DISCUSSION

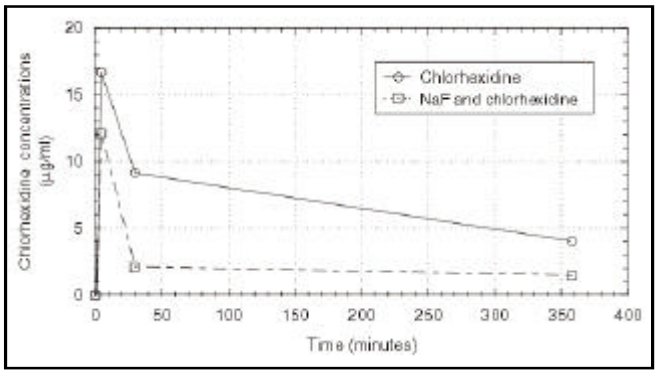
Progresses in science and pharmacology facilitate the access of dentists to a larger number of drugs everyday. However, the professional knows

that for the use of new agents to be beneficial for the patient, more comparative studies should be carefully performed to establish the appropriate therapy strategy.

As previous papers[5-7] focused on the beneficial effect of the fluoride-chlorhexidine therapy to arrest caries and to remineralize incipient lesion, the substantivity was assumed to be satisfactory in relation to the current dose. However, Melo *et al.*[9] (1999) advised that the fluoride-chlorhexidine association was not beneficial, because it reduced the agent concentration.

Brambilla *et al.*[1] (1998) concluded that this associative mouth rinse might be used in caries prevention programs for pregnant women, because it reduces *Streptococcus mutans* scores in the saliva and because it retards colonization in children up to four months.

Diverse studies justified the value of the fluoride-chlorhexidine association on the synergistic effect of the two drugs[3,7]. Due to the low molecular weight of fluoride molecules, they could link to places of difficult access for chlorhexidine and reach *Streptococcus mutans* retention niches and incipient decay lesions, where the pH is low, thus inhibiting acid production with a concomitant prolonged preventive effect[8]. Nonetheless, the fluoride-chlorhexidine association could generate low chlorhexidine concentration in certain retention places, and therefore suppress the potential reservoirs of the drug, such as tongue, mucosa, tonsils and saliva, which is favorable to inhibit the recolonization of treated lesions.



GRAPH 1 - Chlorhexidine concentration (µg/ml) released from bovine enamel fragments impregnated with chlorhexidine gluconate 0.12%, 0.05% sodium fluoride and 0.12% chlorhexidine gluconate.

As verified in this study, the maximum concentration of chlorhexidine observed after 5 minutes was larger for chlorhexidine gluconate than for the association (Graph 1). The initial lower concentration of chlorhexidine in relation to the association was due to the competition between the fluoride ions and the chlorhexidine molecules for the adsorption surface. Other authors[3,7] suggest that there are ionic reactions between fluoride and chlorhexidine, generating a decrease in the concentration of chlorhexidine, and inhibiting the bactericidal effect of the association. However, the fact that chlorhexidine has several positive charges and that it is in larger concentration in the association would neutralize the effect of the drug by fluoride[9].

In agreement with the related literature[7], in the present study it was observed that the substantivity of chlorhexidine was reduced. A strategy to increase the substantivity of fluoride on enamel surface is its association with amine groups, which would link to the negative charges available in teeth and enamel film. This would be interesting because it would prolong fluoride readiness on the dental surface. However, Etemadzadeh *et al.*[4] (1985) showed that the amine fluoride-chlorhexidine association does not inhibit plaque growth. However, the potential to inhibit caries through the use of the fluoride-chlorhexidine association was verified by long-term studies[2,6,7,13,14], even in the treatment of incipient root lesions, with which the association was used[6].

TABLE 1 - Chlorhexidine released (µg/ml) from teeth impregnated with 0.12% chlorhexidine gluconate and from teeth impregnated with 0.05% sodium fluoride and 0.12% chlorhexidine. The values were obtained by Student's *t*-test ($p < 0.05$).

	0.12% chlorhexidine gluconate (Periogard®)			0.05% sodium fluoride with 0.12% chlorhexidine (Duplak®)		
Time (minutes)	5	30	360	5	30	360
Mean	16.68	9.16	4.00	12.14	2.10	1.51
Standard deviation	± 9.43	± 7.94	± 4.58	± 7.08	± 2.29	± 1.56
Variance	88.93	63.18	20.98	50.24	5.25	2.44

The change in the concentration profile of chlorhexidine shown in this study raises new concerns about its interaction with sodium fluoride, and consequently about the benefit of the association of these two drugs.

CONCLUSION

Based on *in vitro* experimental conditions of this work and the results obtained, we can conclude that the fluoride-chlorhexidine association is unfavorable, due to the significant decrease in the substantivity of chlorhexidine.

REFERENCES

1. Brambilla E, Felloni A, Gagliani M, Malerba A, García-Godoy F, Strohmenger L. Caries prevention during pregnancy: results of 30-month study. J Am Dent Assoc 1998;129:871-3.
2. Dolles O, Gjermo P. Caries increment and gingival status during 2 years' use of chlorhexidine- and fluoride-containing dentifrices. Scand J Dent Res 1980;88:22-7.
3. Emilson CG, Krasse B, Westergren G. Effect of a fluoride-containing chlorhexidine gel on bacteria in human plaque. Scand J Dent Res 1976;84:56-62.
4. Etemadzadeh H, Ainamo J, Murtomaa H. Plaque growth-inhibiting effects of an abrasive fluoride-chlorhexidine toothpaste and a fluoride toothpaste containing oxidative enzymes. J Clin Periodontol 1985;12:607-16.
5. Katz S. The use of fluoride and chlorhexidine for the prevention of radiation caries. J Am Dent Assoc 1982;104:164-70
6. Keltjens H, Schaeken M, Van der Hoeven J, Hendriks JC. Caries control in overdenture patients: 18-month evaluation on fluoride and chlorhexidine therapies. Caries Res 1990;24:371-5.
7. Luoma HH, Murtomaa H, Nuuja T, Nyman A, Nummikoski P, Ainamo J, *et al*. A simultaneous reduction of caries and gingivitis in a group of schoolchildren receiving chlorhexidine-fluoride applications. Results after 2 years. Caries Res 1978;12:290-8.
8. Mcdermid AS, Marsh PD, Keevil CW, Ellwood DC. Additive inhibitory effects of combinations of fluoride and chlorhexidine on acid production by *Streptococcus mutans* and *Streptococcus sanguis*. Caries Res 1985;19:64-71.
9. Melo GB, Batista de G, Pinheiro CM, Osório CN, Zardini FA. Potencial de eficácia da associação de clorexidina com flúor. Rev CROMG 1999;5:43-6.
10. Miller WD. The microorganisms of the human mouth: the local and general diseases which are caused by them. Philadelphia: SS White, 1890. Reprint. Basel: Karger, 1973.
11. Oppermann RV, Rölla G. Effect of some polyvalent cations on the acidogenicity of dental plaque *in vivo*. Caries Res 1980;14:422-7.
12. Ostela I, Karhuvaara L, Tenovuo J. Comparative antibacterial effects of chlorexidine and stannous fluoride-amine fluoride containing dental gels against salivary *mutans* streptococci. Scand J Dent Res 1991;99:378-83.
13. Spets-Happonen S, Luoma H, Forss H, Kentala J, Alaluusua S, Luoma AR, *et al*. Effects of chlorhexidine-fluoride-strontium rinsing program on caries, gingivitis and some salivary bacteria among Finnish schoolchildren. Scand J Dent Res 1991;99,130-8.
14. Tenovuo JJ, Häkkinen P, Paunio P, Emilson C G. Effects of chlorexidine-fluoride gel treatments in mothers on the establishment of *mutans* streptococci in primary teeth and the development of dental caries in children. Caries Res 1992;26:275-80.
15. Ullsfoss BN, Ögaard B, Arends J, Ruben J, Rölla G, Afseth J. Effect of a combined chlorhexidine and NaF mouthrinse: an *in vivo* human caries model study. Scand J Dent Res 1994;102:109-12.
16. Wannmacher L, Ferreira MBC. Farmacologia clínica para dentistas. 2nd ed. Rio de Janeiro: Guanabara Koogan S. A.; 1999.
17. Worthington HV, Davies RM, Blinkhorn AS, Mankodi S, Petrone M, DeVizio W, *et al*. A six-month clinical study of the effect of pre-brush rinse on plaque removal and gingivitis. British Dent J 1993;175,322-6.

Recebido para publicação em 24/04/02
Enviado para reformulação em 29/11/02
Aceito para publicação em 07/02/03