Effect of preservatives on reactivity of fluoride with dental enamel

Efeito de conservantes na reatividade do fluoreto com o esmalte dental

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ABSTRACT

***Objectives**: Evaluate the effect of preservatives, usually present in mouthrinse formulations, on fluoride reactivity with enamel.*
***Methods**: Ninety-six bovine enamel slabs (4 x 4 x 2 mm), 48 sound and 48 with caries-like lesions, were longitudinally sectioned through the center. Half of each slab was kept as control and the other one subjected during 10 min to 0.05% NaF solutions containing or not 0.2% methylparaben, 0.02% propylparaben or 0.35% benzoate. Two consecutive layers of enamel were removed from all slabs, by acid etching and fluoride acid extracted was determined with specific electrode.*
***Results**: Fluoride concentration in the half treated was subtracted from that found in the control and expressed as µg F/cm² of enamel surface. Fluoride reactivity with sound enamel was not affected by the preservatives (P > 0.05), but methylparaben increased the reactivity of 0.05% NaF solution with carious one (P < 0.05).*
***Conclusion**: The results suggest that methylparaben may improve fluoride reactivity with enamel, presenting caries-like lesions, but further studies are necessary to evaluate the mechanism.*
***Indexing terms**: fluorides; dental enamel; mouthwashes.*

RESUMO

Objetivos: Avaliar o efeito de conservantes, usualmente presentes em enxagüatórios bucais, na reatividade do fluoreto com o esmalte dental.
Métodos: Noventa e seis blocos de esmalte bonivo (4 x 4 x 2 mm), 48 hígidos e 48 com lesão artificial de cárie, foram seccionados longitudinalmente ao meio. Uma metade de cada bloco foi mantida como controle e a outra reagiu, durante 10 min, com solução de NaF 0,05% contendo ou não metilparabeno 0,2%, propilparabeno 0,02% ou benzoato 0,35%. Duas camadas consecutivas de esmalte foram removidas de todos os blocos por ataque ácido e o fluoreto presente nesse extrato ácido foi determinado com eletrodo específico.
Resultados: A concentração de fluoreto da metade tratada foi subtraída daquela presente nos controles e expressa em µg F/cm² da superfície de esmalte. A reatividade do fluoreto com esmalte hígido não foi afetada pelos conservantes (p>0,05), mas o metilparabeno aumentou a reatividade da solução de NaF 0,05% com o esmalte cariado (p<0,05).
Conclusão: Os resultados sugerem que o metilparabeno pode aumentar a reatividade do fluoreto com o esmalte com lesão de cárie, mas estudos adicionais são necessários para avaliar esse mecanismo.
Termos de indexação: fluoretos, esmalte dentário; anti-sépticos bucais.

INTRODUÇÃO

The decline in dental caries has been explained by the widespread use of fluoride[1], which prevents and controls this disease[2,3], by decreasing dental demineralization or increasing remineralization[4,5]. Fluoride can be delivered by several systems[6] and, among these, mouthrinses are recommended for community programs or self application[7]. Also, they have been suggested for patients at high caries risk[8] and in prevention of root caries[9].

However, the efficacy of fluoridated products in dental caries prevention is closely related with their ability to sustain the salivary fluoride levels[10]. In this way, there is a consensus in the literature that the reactivity of the fluoride present in these products might be modified by change of the

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product pH1[11-13] or interaction with other components of the formulation, such as antibacterial agents[14] or detergents[15,16], which decrease the effectiveness of the active component.

In this context, Tabchoury et al.[17], evaluating fluoridated mouthrinses, found that the products, which presented the highest reactivity with dental enamel, were those containing methylparaben in their composition. Preservatives are used for maintaining pharmaceutical products, cosmetics and foods in aseptical conditions[18], and for maintaining the stability of the products or preventing microbial colonization[19]. Methyl, ethyl and propylparaben are the most commonly used preservatives[20], which are frequently found in commercial hygiene products, but their effect on fluoride reactivity with enamel has not been explored.

Therefore, the aim of this research was to evaluate the effect of methylparaben, propylparaben and benzoate on the fluoride reactivity with sound bovine dental enamel and with artificial caries lesion.

METHODS

Experimental Design

Ninety-six dental slabs were obtained from bovine incisors and caries-like lesions were induced[21] in half of them. The sound and carious enamel slabs were randomly submitted to the following treatment groups (n=12): 0.05% NaF aqueous solutions (Synth, Diadema, Brazil); 0.05% NaF containing 0.2% methylparaben (Synth, Diadema, Brazil); 0.05% NaF containing 0.02% propylparaben (Synth, Diadema, Brazil) or 0.05% NaF containing 0.35% benzoate (Synth, Diadema, Brazil). Previously to the reaction with the solutions, all enamel slabs were longitudinally sectioned through the center into two equal parts. One half of each dental block was kept as control and the other one was subjected during 10 min to one of treatment solution described above. All treatment solutions had their fluoride concentration determined; also, the pH of solutions was determined before and after the reactivity test. From all slabs, two consecutive layers of enamel were removed by acid etching and the fluoride content in the extracts was determined with F specific electrode. Fluoride incorporated was obtained by subtracting fluoride concentration on the half subjected to treatment groups from that found on the control and expressed as μg F/cm^2 of exposed enamel.

Preparation of the enamel slabs

Ninety-six enamel slabs (4 x 4 x 2 mm) were obtained[22] from sound bovine incisor teeth that had been stored in a 2% formaldehyde (Chemco, Campinas, Brazil) solution (pH 7.0) at room temperature for at least 30 days[23]. The dentin was flattened and the enamel surface was polished[22]. During these procedures, the dental slabs were moistened with distilled and deionized water to avoid cracks in enamel. The surfaces of all the slabs were protected with a layer of acid-resistant varnish, except the enamel surface. Then, the slabs were measured with a digital paquimeter (Mitutoyo, Suzano, Brazil) to determine the exposed area (mm^2). Artificial carious lesions were produced in 48 slabs by immersion in a 0.05 M sodium acetate buffer at 37ºC, pH 5.0, 50% saturated in relation to bovine dental enamel[21], in a proportion of 2 mL solution/mm^2 of exposed enamel for 16 h. To prepare this solution, bovine enamel powder (0.074 – 0.105 mm) was kept in a 0.05 M sodium acetate buffer, pH 5.0, (0.25 g/L) for 96 h at 37ºC under agitation. Previously to the reaction with the solutions, all dental slabs, sound and carious, were longitudinally sectioned through the center and two equal parts (4 x 2 x 2 mm) were obtained from each one. One half was kept as a control and the other one was subjected to the respective treatment solution. All sectioned dental slabs were stored in a refrigerated environment (4ºC).

Preparation of the fluoridated solutions

The concentration of fluoride chosen is that found in commercial mouthrinses and the preservatives were chosen based on their use in mouthrinses prepared by dispensing pharmacies and in commercial products for oral hygiene. The concentration of each preservative was based on its limit of solubility in water at room temperature[19].

Fluoride concentration in the solutions was determined after buffering 1:1 with TISAB II (1.0 M acetate buffer pH 5.0, containing 1.0 M NaC^l, 0.4% CDTA and 20g of NaOH/L). The analyses were made in triplicate using a specific electrode ORION 96-06 and an ion analyzer EA 940 (Orion, Boston, USA), previously calibrated with standard fluoridated solutions from 1 to 10 μg F/mL prepared from 100 ppm F standard (Orion 940907). The variation coefficient was lower than 1%. The pH of the solutions was determined before and after the reactivity test, using a glass electrode and a pHmeter (Procyon, São Paulo, Brazil) calibrated with standard buffers pH 4.0 and 7.0 (Orion, Beverly, USA). The pH of the solution containing benzoate was adjusted to 5.95,

which is the mean pH of the other treatment solutions, since its original pH was very acidic (3.43). This change was made with the aim of minimizing the influence of pH on fluoride reactivity with dental enamel[11-13].

Reactivity of fluoride with dental enamel

One half of each dental slab was immersed in the treatment solutions (proportion of 2 mL solution/mm^2 of enamel surface) at room temperature and under slow agitation. After 10 min[17] the slabs were washed for 1 min with distilled and deionized water and stored at 4ºC. The other half control of each slab was stored in the same condition.

Two layers of enamel were consecutively removed from all slabs, control and treated, by acid immersion in 0.25 mL of 0.5 M HCl (Merck, Darmstadt, Germany) for 15 and 30 sec under agitation, followed by buffering with the same volume of TISAB II pH 5.0 modified with additional 20 g of NaOH/L (Merck, Darmstadt, Germany)[24,25] to neutralize the HC^l used in the acid biopsy. The fluoride concentration in the extracts was determined using an ion analyzer ORION EA 940 and an ion specific electrode ORION 96-09[17], previously calibrated with standards of 0.02 to 1.28 µg F/mL (Orion, Beverly, USA). The amount of F in the two layers were combined, fluoride incorporated was obtained by subtracting fluoride concentration in the half dental slab subjected to treatment groups from that found in the control, and expressed as µg F/cm^2.

Statistical Analysis

Fluoride concentration and the pH of the solutions were descriptively analyzed. The results of fluoride per area of exposed enamel were submitted to statistical analyses using the software BioEstat 2.0[26]. The results of fluoride incorporated per area of exposed sound and carious dental enamel were transformed, respectively, by the power of two and square root and submitted to ANOVA followed by Newman-Keuls test. For these analyses the significance limit was established at 5%.

RESULTS AND DISCUSSION

Fluoride concentration in the prepared solutions was close to the expected value (225 µg F/mL) and their initial pH ranged from 5.60 to 6.18 (Table 1). The pH of the solutions increased after the reactivity test, either with sound or carious enamel (Table 1). This may be explained by the fact that enamel is dissolved by the reaction with fluoride and $(PO_4)^{-3}$ and hydroxil ions released might combine with protons from the media, increasing the pH. The lowest increase found was for the benzoate solutions probably due its buffering effect [27].

The preservatives did not affect fluoride uptake by sound enamel, since the differences in comparison with the control were not statistically different (Table 2; $p > 0.05$). However, methylparaben increased the incorporation of fluoride in carious enamel, significantly differing from all the other groups ($p < 0.01$). The increased fluoride reactivity in the presence of methylparaben is in agreement with Tabchoury et al.[17].

The dental slabs with artificial carious lesion treated with fluoridated solution containing methylparaben showed the highest fluoride incorporated. This effect of methylparaben, increasing fluoride uptake by carious enamel, could be explained by the lower initial pH of this solution (5.60) compared with the others (Table 1), considering that

Table 1. Fluoride concentration (µg/mL; means ± sd) in the solutions and their pH before (initial) and after (final) the reaction with enamel.

Solutions	F (µg/mL)	pH		
		Initial	Final	
			Sound Enamel	Carious Enamel
0.05% NaF	224.1 ± 0.9	6.06	6.42	6.30
0.05% NaF and 0.2% methylparaben	234.1 ± 6.4	5.60	6.24	5.94
0.05% NaF and 0.02% propylparaben	235.4 ± 2.6	6.18	6.62	6.52
0.05% NaF and 0.35% benzoate	227.2 ± 3.4	5.95	6.08	6.14

Table 2. Total fluoride incorporated (µg/cm^2; means ± sd) in sound and carious enamel according to the treatments (n= 12).

Treatments	Fluoride incorporated (µg/cm^2)	
	Sound	Carious
0.05% NaF	0.93 ± 1.50^a	0.34 ± 0.53^b
0.05% NaF and 0.2% methylparaben	0.17 ± 0.26^a	2.58 ± 1.69^a
0.05% NaF and 0.02% propylparaben	0.23 ± 0.23^a	0.24 ± 0.45^b
0.05% NaF and 0.35% benzoate	0.31 ± 0.45^a	0.20 ± 0.32^b

Means of the treatments in sound and carious enamel followed by distinct letters differ statistically ($P < 0.05$)

fluoride reactivity with enamel is inversely dependent of the medium pH[11-13]. In this context, the decrease in pH of fluoridated solutions was followed by an increase in fluoride uptake by enamel[28, 29]. Another evidence for a pH-effect is that the solution containing methylparaben, which reacted with carious enamel, presented the lowest final pH (5.94) compared with the others (Table 1). This suggests that, probably, methylparaben presented a buffering effect, maintaining a low pH during the reaction. In this way, the maintenance of low pH in methylparaben solution might be responsible for the higher uptake of fluoride by the carious enamel.

However, the difference of pH, after the reaction, between methylparaben and benzoate solution is only 0.20 units, suggesting that the findings could be explained by other factors. Likewise, it has been described that some compounds, such as surface agents[30, 31] and surfactants[32], can increase the reactivity of fluoride. As methyl and propylparaben show similar chemical structures, this effect would also be observed in the group treated with propylparaben, if the same concentration of methylparaben were adopted. However, in order to increase the concentration of propylparaben it would be necessary to add another solvent to the solution, since the concentration used for propylparaben was the maximum considering its solubility in water. Thus, in this case, a control group should be necessary in order to evaluate the single effect of the solvent in the reactivity of fluoride with dental enamel, and this is the aim of a new research that is being conducted. Also, in this study, the methylparaben concentration in treatment solution is 10 times higher than the concentration of propylparaben, which a strong evidence for a pH buffering effect. Thus, this effect of methylparaben, increasing the reactivity of fluoride with enamel, should be further evaluated using buffered solutions to exclude the pH effect from a possible one related to its chemical structure.

CONCLUSION

In conclusion, the results suggest that methylparaben present in a fluoridated solution may improve fluoride reactivity of the formulation with enamel, presenting caries-like lesions. Nevertheless, further studies are required to better understand this effect.

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