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EFFECT OF FLUORIDATED VARNISH AND SILVER DIAMINE FLUORIDE SOLUTION ON ENAMEL DEMINERALIZATION: pH-CYCLING STUDY

EFEITO DO VERNIZ FLUORETADO E DA SOLUÇÃO DE DIAMINO FLUORETO DE PRATA NA DESMINERALIZAÇÃO DO ESMALTE: ESTUDO UTILIZANDO MODELO DE CICLAGEM DE pH

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ABSTRACT

Objective: In the present investigation, the anticariogenic effect of fluoride released by two products commonly applied in infants was evaluated. Methods: Bovine sound enamel blocks were randomly allocated to each one of the treatment groups: control (C), varnish (V) and diamine silver fluoride solution (D). The blocks were submitted to pH cycles in an oven at 37°C. Next, surface and cross-sectional microhardness were assessed to calculate the percentage loss of surface microhardness (%SML) and the mineral loss (ΔZ). The fluoride present in enamel was also determined. Results: F/Px10^{-3} (ANOVA, $p<0.05$) in the 1st layer of enamel before pH-cycling were (C, V and D): 1.61^{a}; 21.59^{b} and 3.98^{c}. The %SMH (Kruskal-Wallis, $p<0.05$) were: -64.0^{a}, -45.2^{b} and -53.1^{c}. %ΔZ values (ANOVA, $p<0.05$) were: -18.7^{a}, -7.7^{b} and -17.3^{a}. Conclusion: The data suggested that the fluoride released by varnish showed greater interaction with sound enamel and provided less mineral loss when compared with silver diamine solution.

Uniterms: Enamel; Demineralization; Fluoride; Varnish; Dental caries, prevention and control.

RESUMO

Objetivos: Este trabalho avaliou o efeito anticariogênico do flúor liberado por dois produtos comumente aplicados em crianças. Métodos: Para isto, utilizaram-se blocos de esmalte de dentes bovinos distribuídos aleatoriamente em três grupos de tratamento: controle (C), verniz fluoretado (V) e solução de diamino fluoreto de prata (D). Os blocos foram submetidos à ciclagem de pH a 37ºC. Após, realizou-se o teste de microdureza de superfície (para o cálculo da % da perda de microdureza de superfície - %SML) e em secção longitudinal do esmalte (% de alteração da área mineral - %ΔZ) e a determinação do flúor presente no esmalte (F/P x 10^{-3}). Resultados: As concentrações de flúor (ANOVA, $p<0,05$) na 1ª camada do esmalte, antes da ciclagem de pH, foram (C, V e D): 1,6^{a}; 21,6^{b} e 4,0^{c}. Os resultados de %SML (Kruskal-Wallis, $p<0.05$) foram: -64,0^{a}, -45,2^{b} e -53,1^{c}. Os valores de %ΔZ (ANOVA, $p<0.05$) foram: -18,7^{a}, -7,7^{b} e -17,3^{a}. Conclusão: Os dados sugerem que o flúor liberado pelo verniz apresentou maior interação com o esmalte promovendo menor perda mineral quando comparado à solução de diamino de prata.

Unitermos: Esmalte dentário; Fluoreto; Verniz; Cárie dentária, prevenção e controle.

INTRODUCTION

In the present conception, the effect of fluoride on tooth decay control is attributed to its constant presence in the oral cavity, which contributes positively by working directly on the demineralization/remineralization phenomena. Professionally applied topical fluorides are recommended for those individuals who present moderate to severe caries activity, and aims at preventing and/or arresting new or recurrent dental cavities, since supplementary application is able to reduce the carious increment, which is around zero[16]. Professionally applied topical fluorides are used restrictively in dental offices and may be supplied in solutions, gels, varnishes, foams or prophylactic pastes[11].

The Brazilian assistance programs recommend the use of silver diamine fluoride solutions (cariostatics)[24] or sodium fluoride varnishes[4] for children aged 0 to 3 years old at moderate or high activity of tooth decay. Both are suggested as fluoride supplement for preventing enamel caries mainly because of their cariostatic effect, easy application and preventive properties[4,24,25].

The use of these topical products has been supported by several investigations that have evaluated the enamel resistance to acid etching by increasing fluoride incorporation in the enamel or by decreasing the enamel solubility in acids. However, this outcome is not correlated with clinical findings because the effect on caries dynamics has not been studied[26]. Moreover, there are no parameters comparing the use of those two products either concerning enamel resistance to demineralization under conditions that simulated the development of caries or the effect of silver diamine fluoride on sound enamel.

Thus, the aim of this investigation was to study the effect of the application of a fluoridated varnish or a silver diamine fluoride solution on fluoride uptake by sound enamel, and enamel resistance to demineralization in an in vitro cariogenic challenge.

MATERIAL AND METHODS

Experimental design

Enamel blocks (4X4 mm) were obtained from bovine incisor teeth stored in formaldehyde at 2%, pH 7.0, at room temperature[6]. The blocks were serially polished and flattened for initial surface microhardness (SMH) assessment. Those blocks with microhardness ranging from 326 to 344 KHN (Knoop hardness) units were selected and randomly distributed into 3 groups with 24 specimens each. One group was used as control (C); in the other groups, either a fluoridated varnish (V) or a silver diamine fluoride solution (D) was applied to the enamel. Twelve blocks of each group were submitted to pH-cycling solutions and the others were kept in humid environment until analysis. Half of each block submitted to pH-cycling was designated for surface and cross-sectional microhardness (CSMH) determination, and the other half, as well as those blocks not submitted to pH-cycling, were designated for enamel fluoride concentration analysis.

Treatments and pH-cycling

A thin layer of fluoridated varnish (Duraphat 2.26% F-pH 7.0, Woelm & Pharma Co., Eschwege, Germany) was applied with a brush to the enamel blocks of the "V" group. After 24 hours, the varnish was removed carefully with a surgical blade. Removal was completed with cotton swabs soaked in acetone[2,19]. Then, the blocks were washed with deionized water for 1 minute. Silver diamine fluoride (Safluoride di Walter at 30% - pH 9.0, Polidental, Rio de Janeiro, RJ, Brazil) was applied with a cotton swab to the enamel blocks of the "D" group for 2 minutes. After application, the blocks were washed with a flow of deionized water for approximately 30 sec and lightly dried with absorbent paper. The enamel blocks of groups C, V and D that were not submitted to cariogenic challenge were stored in humid environment until the end of pH-cycling.

Twelve blocks from each group were submitted to a pH-cycling model simulating a high caries challenge for 7 days, basically according to Vieira et al[23]. The blocks were kept in demineralizing solution (2.0 mmol L^{-1} calcium, 2.0 mmol L^{-1} phosphate in 0.075 mol L^{-1} acetate buffer, 0.02 µm F/mL, pH 4.7) for 3 h (35.5 mL per block), and in a remineralizing solution (1.5 mmol L^{-1} calcium, 0.9 mmol L^{-1} phosphate, 150 mmol L^{-1} KCl in 0.1 mol L^{-1} Tris buffer, 0.03 µm F/mL, pH 7.0) for 21 h (17.75 mL per block).

Microhardness determination

After pH-cycling, surface microhardness of the enamel blocks from the C, V and D groups was measured again (SMH_1). Five indentations spaced 100 µm from each other in relation to the baseline were made using the microhardness tester Shimadzu HMV-2000 (Shimadzu Corporation, Kyoto, Japan). The percentage loss of surface microhardness (%SML) was calculated ($\%SML = [[SMH_1 - SMH]/SMH]x100$)[23]. Next, the blocks were longitudinally sectioned at the midline. Half of each block was used for cross-sectional microhardness (CSMH) determination and the other half for enamel fluoride concentration analysis. For cross-sectional microhardness tests, one of the halves of each block was embedded in acrylic resin so that the sectioned enamel blocks could be exposed and polished. The indentations were made at 20, 40, 60, 80, 100, 200 and 300µm from the outer enamel surface. CMHS values were converted to mineral content (volume % mineral) using the relation: mineral content = 4.3 ($\sqrt{KHN}$) + 11.3[7]. Integrated area (% min. vol. x µm) of sound enamel (Z) and after pH-cycling (Z_1) were calculated[1,14,20]. The percentage change of integrated mineral loss [$\%\Delta Z = ((Z_1 - Z)/Z)x100$] was calculated.

Enamel fluoride concentration

Three thin enamel layers were sequentially removed by immersion of half of each block in 0.5 mL of 0.5 mol L^{-1} of HCl for 15, 30 and 60 seconds under constant agitation[23]. An equal volume of TISAB II, pH 5.0 (modified with 20g NaOH/L) was added to each solution containing the dissolved enamel layer. Fluoride measurements (μg F/cm^2) were taken using an ion-selective electrode Orion 96-09 and an ion analyzer Orion 720A (Orion Research, Inc., Beverly, MA, USA). The fluoride concentration in each layer was presented as the ratio of fluoride (μg F/cm^2) and phosphate (μg P/cm^2) values, which was determined by the method of Fiske and Subbarrow[8].

Statistical analysis

Data obtained from cross-sectional microhardness (%ΔZ) and enamel fluoride concentration measurements (ratio of fluoride and phosphate) were submitted to the analysis of variance and to the Tukey test at 5% of significance. Heterogeneous variances were detected among the results of the SMH, SMH_1 and %SML by Shapiro-Wilks normality test. Thus, the Kruskal-Wallis tests – non-parametric multiple comparison tests – were applied to distinguish significant differences among the treatments at the level of 5%[3].

RESULTS

The results obtained for the different groups and treatments with regard to SMH, SMH_1 and %SML are shown in Table 1. The values of baseline microhardness (SMH) observed in the control (C), fluoridated varnish (V) and silver diamine fluoride (D) groups were similar ($p>0.05$). The SMH_1 and %SML values of the enamel blocks treated with fluoridated varnish (V) were higher and lower, respectively, than those obtained in the silver diamine fluoride (D) and control (C) groups ($p<0.05$). Furthermore, enamel blocks treated with silver diamine fluoride solution presented higher SMH_1 and lower %SML values in relation to the control group ($p<0.05$). Concerning mineral loss (%ΔZ), the values obtained in the “V” group were significantly lower ($p<0.05$) than those in the “D” and “C” groups, which were similar ($p>0.05$).

With regard to the F/P ratio at the first and second layers before pH-cycling, all values observed in the different treatment groups were higher than those obtained in the control group (C). The values of F/P ratio observed in the group “V” were higher than those observed in the groups “C” and “D” before and after pH-cycling, and showed significant difference at the level of 5%. The distribution of fluoride concentrations throughout the enamel layers and their statistical significance are illustrated in Table 2.

DISCUSSION

Many in vitro and in vivo studies have been conducted to define the desirable fluoride therapy for dental decay prevention[12]. High frequency application with low concentrations of fluoride agents has been considered the most beneficial treatment regime. However, in situations of high risk of caries, the association of a method that employs high concentration of fluoride such as the professionally applied products has been recommended[16]. In infants, both fluoridated varnish and silver diamine fluoride have been indicated and used due to their easy application and safety on enamel demineralization, even though diamine has been designed to be applied to dentin. However, comparison between these two fluoride agents on enamel demineralization has not been clearly established.

The results of the present study showed that the enamel blocks treated with fluoridated varnish (V) showed better results regarding %SML, mineral loss (ΔZ) (Table 1) when compared with the control (C) and the silver diamine fluoride (D) groups. The group D, in turn, showed a %SML value lower than that of the control group (C).

However, mineral loss for group “D” was 2.2 times higher when compared to group “V”. The silver diamine fluoride was not effective to decrease mineral loss (%ΔZ) in comparison with the control group. The findings of %ΔZ do not confirm the SMH results regarding the effect of silver diamine fluoride. This difference may be due to the amount and the type of F compound formed on the surface. The silver diamine fluoride showed a lower F formed (before pH cycling) and released (after pH cycling). Despite the F from silver diamine was able to reduce the %SML, it was not able to decrease the mineral loss from the subsurface. This may be the reason why fluoride varnish is more efficient to delay lesion progression when compared to silver diamine[18].

Even though silver diamine provides more soluble fluoride (23.6%) in comparison with varnish (15%)[2], the results from F in the enamel showed that higher amounts of

TABLE 1- Analysis of the enamel blocks according to groups/treatments (means ± sd; n=12)

Treatments	Analysis			
	SMH	SMH_1	%SML	%ΔZ
Control	^{A}338.2 ± 1.8^a	^{B}121.8 ±20.1^a	-64.0 ± 4.9^a	-18.7 ± 2.7^a
Varnish	^{A}339.8 ± 2.5^a	^{B}186.2 ±16.9^b	-45.2 ± 4.1^b	-7.7 ± 3.1^b
Diamine	^{A}337.2 ± 3.2^a	^{B}158.0 ±10.7^c	-53.1 ± 1.8^c	-17.3 ± 2.3^a

Mean values followed by different letters are statistically different at 5%. Capital letters show difference between SMH and SMH_1, and lower case letters among groups. SMH: initial surface microhardness. SMH_1: surface microhardness after pH-cycling. %SML: percentage of surface microhardness loss. %ΔZ: percentage change of integrated mineral loss.

F are formed and retained in the enamel after treatment with fluoride varnish in all removed layers, except for the third layer of F retained. This fluoride may be released during the cariogenic challenge and may spread into the enamel and reduce the caries lesion progress, propitiating the re-precipitation of less soluble calcium phosphate[5]. This may be confirmed by a higher F formed / F retained ratio in the "V" group in all enamel layers removed by acid biopsy in comparison with the "D" and "C" groups (Table 2). These can be correlated to the %ΔZ: the silver diamine failed to inhibit caries progression.

The amount of F formed in the enamel depends on the F concentration and the pH of the product applied and how long it remains in contact with the enamel. Thus, the better results observed for the group "V" may be related to a longer contact period of the varnish with the enamel and the basic pH (9.0) presented by silver diamine fluoride.

For the silver diamine group, the F present in enamel did not differ from the control group in many removed layers (Table 2). In the third layer after pH cycling (F retained), the silver diamine showed lower amount of F when compared with the control group. This demonstrates that the fluoride released from silver diamine is not able to penetrate in depth in the enamel. Even though fluoride concentration in enamel not always provides conclusive information with regard to the products tested[22], these observations can be correlated with mineral loss (ΔZ). The products formed from silver diamine and their interaction with calcium phosphate within the lesion were not effective to decrease caries progression in comparison with the control group.

The worse performance for group D may be attributed to the formation and dissolution of silver phosphate and silver proteinate and re-precipitation of silver chloride, silver oxide and metallic silver crystals during pH-cycling[13]. Silver may have interfered with the formation of fluoridated deposits and formed silver phosphate, which may compete with fluoride. In contrast, the main product formed after fluoride varnish application is the calcium fluoride, which may act as a fluoride reservoir. During acid challenge, the calcium fluoride dissolves and the F is released to effectively promote remineralization.

The silver fluoride products are more often used in dentin caries, which present a greater amount of protein substrate, carbonates and phosphates for the reaction. On the other hand, the enamel is short of these substrates in comparison with dentin, which may have decreased the silver diamine fluoride reactivity. This may contribute to the differences observed in the results of the present study[10]. In addition, these results also suggested that it should be convenient to use silver diamine fluoride more frequently than fluoridated varnish to compensate for its lower reactivity. However, this should be evaluated by in situ and further clinical trials.

CONCLUSION

Based upon the experimental conditions of this study, we concluded that fluoridated varnish was more effective to reduce both the enamel surface demineralization and caries lesion depth than silver diamine fluoride solution.

TABLE 2- Mean ratio of fluoride and phosphate values (F/P x 10^{-3}) before and after pH-cycling in the removed enamel layers (means ± SD; n = 12)

GROUPS		Control	Varnish	Diamine
Before pH-cycling				
(F formed)	1st layer	^{A}1.6 ± 0.3^{a}	^{B}21.6 ± 4.0^{a}	^{C}4.0 ± 0.5^{a}
	2nd layer	^{A}1.3 ± 0.3^{a,b}	^{B}13.2 ± 2.4^{b}	^{C}2.4 ± 0.5^{b}
	3rd layer	^{A}0.9 ± 0.2^{b,c}	^{B}7.4 ± 1.5^{c}	^{A}1.2 ± 0.3^{c,e}
After pH-cycling				
(F retained)	1st layer	^{A}2.0 ± 0.3^{a}	^{B}3.4 ± 0.9^{d}	^{A}1.7 ± 0.4^{d}
	2nd layer	^{A}1.4 ± 0.2^{a,b}	^{B} 1.5 ± 0.4^{e}	^{A}0.9 ± 0.1^{e}
	3rd layer	^{A}0.6 ± 0.1^{c}	^{A}0.7 ± 0.1^{f}	^{B}0.4 0.1^{f}
Ratio F formed /				
F retained	1st layer	0,8	6,3	2,3
	2nd layer	0,9	8,8	2,6
	3rd layer	1,5	10,6	3,0

Mean values followed by different letters are statistically different at 5%. Capital letters show differences between the treatment groups; lower case letters show differences between the enamel layers removed within the same group.

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