

ANTICARIOGENIC POTENCIAL OF ACIDULATE SOLUTIONS WITH LOW FLUORIDE CONCENTRATION

POTENCIAL ANTICARIOGÊNICO DE SOLUÇÕES ACIDULADAS COM BAIXA CONCENTRAÇÃO DE FLÚOR

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ABSTRACT

O bjetives: The aim of this study was to verify the anticariogenic effect of acidulate solutions with low NaF concentration, using pH-cycling model and bovine enamel. Material and methods: Enamel blocks were submitted to the surface microhardness (SMH) test and randomly divided in 12 experimental and one placebo groups. The blocks were submitted to pH cycling for 7 days, with daily applications once/day of 0.05% NaF and 0.1% NaF and twice/day of 0.02% NaF solutions. Four different pH: 4.0, 5.0, 6.0 and 7.0 were used. Next, SMH test was again used to determine the surface microhardness percentage change (%SMH). Data obtained for %SMH were homogeneous and passed through variance analyses and Tukey's test (5%) as far as fluoride concentrations and pH. Results: The results showed that pH influenced %SMH in 0.02% NaF and 0.05% NaF solutions with pH 4.0, which had less mineral loss compared to pH 7.0 ($p < 0.05$). The 0.02% NaF - pH 4.0, and 0.05% NaF - pH 7.0 groups showed similar results ($p > 0.05$). A dose-response relationship was observed among the tested solutions, with better anticariogenic effect for the 0.1% NaF solution. Conclusion: The results suggest that the addition of citric acid to acidulate mouth rinses reduce mineral loss.

Uniterms: Dental enamel, hardness; Sodium fluoride, administration & dosage; Dental caries, prevention & control.

RESUMO

O bjetivo: O objetivo do presente estudo foi verificar o efeito anticariogênico de soluções aciduladas com baixa concentração de flúor, usando um modelo de ciclagem de pH e esmalte bovino. Material e Método: Blocos de esmalte foram polidos, submetidos ao teste de microdureza de superfície (SMH) e divididos aleatoriamente em 12 grupos experimentais e um placebo. Os blocos foram submetidos à ciclagem de pH por 7 dias e a aplicações diárias de soluções de NaF a 0,05% e 0,1% (1x) e de 0,02% (2x), com pHs 4,0, 5,0, 6,0 e 7,0. A seguir, determinou-se a SMH novamente para o cálculo da porcentagem de alteração da microdureza de superfície (%SMH). Os dados de %SMH foram homogêneos e submetidos a análise de variância e teste de Tukey (5%) considerando as concentrações de flúor e pH. Resultados: O pH influenciou a %SMH nas soluções de NaF 0,02% e 0,05% com pH 4,0, no qual a perda mineral foi menor quando comparado ao pH 7,0 ($p < 0,05$). Os grupos das soluções de NaF 0,02% - pH 4,0, e de NaF 0,05% - pH 7,0 mostraram resultados similares ($p > 0,05$). A relação dose-resposta foi observada entre as soluções testadas, com melhor efeito anticariogênico para a solução de NaF 0,1%. Conclusão: Os resultados sugerem que a acidificação das soluções com baixa concentração de fluoreto reduz a perda mineral.

Unitermos: Esmalte dentário, dureza; Fluoreto de sódio, administração & dosagem; Cárie dentária, prevenção & controle.

INTRODUCTION

Fluoride use is quite relevant, mainly because it increases the saliva ability of mineral replacement of teeth during the post-eruptive phase in 2 to 4 times⁸. Topical fluorides are known as efficient agents for dental caries prevention^{3,14} and many *in vitro* and *in vivo* researches were accomplished to define their efficacy. The use of fluoride has been recommended in constant frequency and low levels for better effect². Studies show that the use of low-fluoride solutions can reduce enamel demineralization and increase remineralization^{1,15}.

Different concentrations of fluoride solutions and dentifrices have been studied because of the high index of dental fluorosis. According to the literature, the fluoride amount regarded as safe ranges from 0.05 to 0.07 mg F/Kg weight/day⁵. Some studies indicate that young children ingest a large amount of toothpaste during brushing^{17,18}. Toddlers tend to swallow one third or more of the amount of dentifrice placed on toothbrushes¹⁶. Oral health promotion programs for toddlers (Baby Clinics) have been using topical solutions with low fluoride concentrations in the dental office (0.2% NaF/ bi-monthly) and at home (0.02% NaF/ day) instead of fluoride dentifrice as a safety measure^{10,19,21}. The use of 0.02% NaF solutions does not lead to risk of chronic intoxication in children, even if combined with other fluoride sources to which these patients may be exposed¹³. However, the use of 0.02% NaF solution is not based on clinical researches⁹. Besides that, an *in vitro* study did not confirm the anticariogenic effect of this concentration⁷. Solutions with other fluoride concentrations have been suggested, but without scientific confirmation. Thus, is very important to establish a dose-response relationship before indicating fluoride products.

According to Bijella, et al.⁴(1994), the reduction of pH of fluoride solutions increases the anti-metabolic effect of fluoride in dental plaque. Delbem and Cury¹¹(2002) observed that acidulated products promoted better fluoride uptake and were more efficient to reduce mineral loss when compared to neutral products. Thus, to compensate the low reactivity, a more frequent use of neutral products has been suggested.

Therefore, it would be important to verify if pH alterations would improve the anticariogenic effect of low-fluoride solutions for utilization by 0 to 3 year-old children, for achievement of the dose-response risk relationship. The aim of this study was to verify the anticariogenic effect of 0.02%, 0.05% and 0.1% NaF solutions and the influence of pH of these solutions, (pH 4.0, 5.0, 6.0 and 7.0), using a pH-cycling model and bovine teeth.

MATERIAL AND METHODS

Experimental design

One hundred and thirty enamel blocks (4x4 mm) achieved from bovine incisors had their enamel surfaces sequentially polished, allowing selection of blocks by determination of

the initial surface microhardness (SMH). The study design was random, and the blocks were divided into 13 groups with 10 specimens each: placebo solution (deionized water), 0.02%, 0.05% and 0.1% NaF solutions. All fluoride solutions were prepared in four different pH (4.0, 5.0, 6.0 and 7.0) using citric acid. The enamel blocks were submitted to a pH cycling model for analysis of the dose-response effect of fluoridated solutions. The placebo and 0.02% NaF solutions were applied twice a day; 0.05% and 0.1% NaF solutions were applied once a day. After this, the blocks were submitted to a final surface microhardness analysis.

Analysis of the fluoride concentration in solutions

After the codification of the products, 1 mL of each solution (0.02%, 0.05% and 0.1% NaF solutions in pH 4.0, 5.0, 6.0 and 7.0) was pipetted in 100 mL polypropylene volumetric balloons and the volume was completed with deionized water. This step was repeated 3 times. Then, three samples of 1 mL of each diluted solution were pipetted in polystyrene vials (J 10), with a total of nine samples of each product. Fluoride measurements were performed using an ion-selective electrode BN 9609 (Orion Research) and an ion analyzer 290A (Orion Research). Previously, a calibration curve was made with fluoride standard solutions prepared in different concentrations: 0.125 to 2.0 µg F/mL for the 0.02% NaF solution; 0.25 to 4.0 µg F/mL for the 0.05% NaF solution and 0.5 to 8.0 µg F/mL for the 0.1% NaF solution. Fluoride was measure after mixing 1 mL of the diluted sample and 1 mL of TISAB II, in constant and light agitation^{12,14}.

Demineralizing and remineralizing cycling

The blocks were submitted during 7 days at 37°C to a pH-cycling model, according to Vieira, et al.²⁰ (2005), altering demineralizing sessions (2.0 mmol L⁻¹ calcium and phosphate in 75 mmol L⁻¹ acetate buffer, pH 4.7; 0.04 µg F/mL, 2.2 mL/mm²) for 6 h, and remineralizing (1.5 mmol L⁻¹ calcium, 0.9 mmol L⁻¹ phosphate, 150 mmol L⁻¹ KCl in 0.02 mol L⁻¹ cacodylic buffer, pH 7.0; 0.05 µg F/mL, 1.1 mL/mm²) for 18h.

The treatment regimen consisted of 30 seconds soak in 2 mL/block of placebo and 0.02% solutions under agitation on a rotatory shaker, before the solution changes from DE to RE and from RE to DE (twice a day). For the 0.05% and 0.1% solutions, the treatments were realized before the solution changes from RE to DE (once a day). Deionized water rinses were done between every step. In the last two days the blocks were kept in remineralizing solution.

Microhardness Analysis

Enamel microhardness measurements were realized as described by Delbem and Cury¹¹ (2002) and performed before (SMH) and after (SMH₁) pH-cycling. A Shimadzu HMV-2000 microhardness tester (Shimadzu Corporation, Kyoto, Japan) Knoop diamond under a 50g load for 10 seconds coupled to a CAMS-WIN software (New Age Industries, USA) was used. The percentage change of surface microhardness (%SMH) was calculated [%SMH = ((SMH₁ - SMH)/SMH)x100]²⁰.

STATISTICAL ANALYSIS

Since the % SMH values were homogeneous, data obtained were submitted to the variance analyses and Tukey’s test (5%) considering fluoride concentrations and pH, using the GMC Statistical Software⁶. Mean, percentage of the variation coefficient and confidence interval (95%) were determined for fluoride values (ppm) in the solutions.

RESULTS

Analysis of fluoride concentration in solutions

Table 1 shows the fluoride content of the solutions according to the different concentrations and pH used in this study. All fluoride products presented means of total fluoride content within the confidence interval of 95%. The variation coefficient obtained, referring to the concentration defined for each solution, was under 10% in all solutions.

Microhardness Analysis

Table 2 shows the %SMH according to pH (4.0, 5.0, 6.0 and 7.0) and NaF concentrations (0.02%, 0.05% and 0.1%). Less mineral loss was observed in the 0.02% NaF solution of pH 4.0, compared to other solutions with same concentration and different pH. Similar data were presented by 0.05% NaF solutions. There was no difference related to pH alteration (p>0.05) of the 0.1% NaF solution. Analyzing the effect of the fluoride concentration in solutions, less mineral loss was observed in the 0.1% NaF solution (p<0.05). A negative correlation was found among the different fluoride concentrations and %SMH (r= - 0.98; p= 0.021).

DISCUSSION

In vitro studies using pH cycling model should allow verification of dose-response relationship of fluoridated products. Therefore, to validate the results of the present

research, the model must provide this verification^{3,20}. The present results showed that the model employed to simulate the *in vivo* cariogenic challenge allowed observation of the dose-response relationship related to %SMH and fluoride concentration of the solutions. This validates the obtained results related to the effect of acidification of fluoride solutions.

Fluoride solutions were dosed before pH-cycling and presented means of total fluoride concentration within the 95% confidence interval. The variation coefficient, having the defined concentration of each solution as reference (Table 1), was lower than 10%. This variation is allowed by the Agência Nacional de Vigilância Sanitária (National Sanitary Surveillance Agency) – ANVISA¹ and is similar to fluoridated mouthrinses commercially available in Brazil, according to Delbem, et al.¹⁴ (2003).

Surface microhardness analysis (SMH) is a sensitive and reproducible method to verify the initial phase of the caries

TABLE 2- %SMH values according to the fluoride concentrations and pH of the solutions (mean ± sd; n=10)

pH	Fluoride concentrations (% NaF)		
	0.02	0.05	0.1
4.0	48.3 ±4.4 ^a	41.9 ±8.5 ^{c,d}	38.9 ±2.6 ^d
5.0	53.6 ±3.7 ^b	49.0 ±2.8 ^a	37.4 ±11.1 ^{c,d}
6.0	54.1 ±4.0 ^b	49.1 ±7.0 ^{a,b}	41.8 ±3.0 ^{c,d}
7.0	58.5 ±6.3 ^b	48.6 ±6.3 ^a	44.7 ±1.8 ^{a,c}
Placebo		81.0 ±3.3 [*]	
%SMH	53.6 ±5.8 ^A	47.3 ±7.0 ^B	40.7 ±6.4 ^C

Means followed by distinct letters are statistically different (p<0.05). Mean followed by asterisk is statistically different to others groups (p<0.05). Capital letters show difference related to the effect of the concentration of fluoride.

TABLE 1- Means (n=18) of fluoride content (ppm) of the experimental fluoride solutions according to their concentration and pH

pH	Fluoride concentration (%NaF – ppm F)		
	0.02 – 90	0.05 – 225	0.1 – 450
4.0	89.6 (-0.4) [*] (88.6 to 90.5) ^{**}	218.6 (-2.8) (216.2 to 221.1)	450.9 (2.2) (457.1 to 462.1)
5.0	91.9 (2.1) (91.3 to 92.6)	225.6 (0.3) (223.8 to 227.4)	458.0 (1.8) (455.8 to 460.3)
6.0	83.9 (-6.7) (83.6 to 84.3)	225.8 (0.4) (224.8 to 226.8)	439.0 (-2.4) (437.1 to 440.8)
7.0	96.2 (6.8) (95.8 to 96.6)	224.3 (-0.3) (223.8 to 224.9)	426.1 (-5.3) (424.7 to 427.4)

* percentage of the variation coefficient ; ** confidence interval (95%).

process³. Also, White²² (1987) found a strong correlation ($r^2=0.94$; $p<0.01$) using SMH or microradiographs to investigate the remineralization of incipient carious lesions. As observed above, the effect of different concentration and pH of solutions was verified on the enamel surface, proving the sensitivity of the method and that interactions occurred among the enamel and solutions on the enamel surface.

SMH values showed that acidification of the solutions promoted a better fulfillment for 0.02% and 0.05% NaF solutions at pH 4.0 (Table 2). Acidification of the 0.1% NaF solution did not show significant effect when compared to other concentrations (Table 2). Reduction in enamel mineral loss was possible only at a very acidic pH, under the critical pH of enamel (5.5). However, citric acid, which is a weak acid, was used as acidulating agent, because it is comestible, so that it can be ingested without health damage, an important attribute when consumed by babies. Therefore, it promotes less enamel demineralization and ion release (calcium and phosphate) in order to allow reaction with the fluoride of the solutions. This reaction is important for formation of calcium fluoride, which is responsible for the anticariogenic effect of topical products¹¹.

An important finding was that, at pH 4.0, the 0.02% NaF promoted similar results when compared to the 0.05% solution at pH 7.0, 6.0 and 5.0. Thus, the use of this experimental solution in babies can be effective for daily use with a lower risk of development of dental fluorosis. This is due to the amount of fluoride applied, and the quantity that could be ingested from the use of the 0.02% NaF solution twice a day would be lower compared to utilization of the 0.05% NaF solution only once a day, what reinforces the possibility of its use. However, this study did not evaluate the influence from saliva as a buffering element capable of excluding the effect observed in the *in vitro* study. Therefore, it would be important to test the formulations able to keep this effect without being inactivated by the buffering capacity of saliva.

The results of the present study suggest a dose-response relationship among the experimental solutions tested, with better anticariogenic effect for the 0.1% NaF solution. Acidification of the experimental solutions promoted better anticariogenic effect only at pH 4.0, for the 0.02% and 0.05% NaF solutions.

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