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DENTIFRÍCIO DE BAIXA CONCENTRAÇÃO DE FLUORETO: EFEITO ANTICÁRIE E MECANISMOS ENVOLVIDOS

Dissertação apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do Título de Mestre em Odontologia, Área de concentração em Cariologia.

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PROF. DR. FERNANDO BORBA DE ARAÚJO

PROFa. DRa. MARINÊS NOBRE DOS SANTOS UCHOA

“Dies santificatus illuxit nobis...”

“Deus santificado ilumina-nos...”

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dove è offesa che io porti il perdono,
dove è discordia che io porti l'unione,
dove è dubbio che io porti la fede,
dove è errore che io porti la verità,
dove è disperazione che io porti la speranza,
dove è tristezza che io porti la gioia,
dove sono tenebre che io porte la luce.
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ad essere compreso quanto a comprendere,
ad essere amato quanto ad amare,
poiché donando si riceve,
perdonando si é perdonati,
morendo si risuscita a vita eterna.

(atribuída a San Francesco d'Assisi)

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onde houver ofensa, que eu leve o perdão;
onde houver discórdia, que eu leve a união;
onde houver dúvida, que eu leve a fé;
onde houver erro, que eu leve a verdade;
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onde houver tristeza, que eu leve a alegria;
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consolar, que ser consolada;
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RESUMO

A eficiência anticárie dos dentifrícios fluoretados contendo 1000-1500 µg F/g está bem estabelecida, porém eles têm sido considerados fator de risco para fluorose dental. Para reduzir esse risco, dentifrícios contendo baixa concentração de fluoreto (F) (500-550 µg F/g) têm sido recomendados, mas sua eficiência anticárie ainda não foi demonstrada. Assim, o objetivo deste trabalho foi: 1. comparar a disponibilidade de F na saliva após utilização de dentifrício de baixa concentração de F (BC, 500 µg F/g, NaF), ou dentifrício de concentração convencional (CC, 1100 µg F/g, NaF), seguida ou não de enxágüe; e 2: avaliar *in situ* o potencial anticariogênico desses dentifrícios, estudando o efeito do F disponível no biofilme dental após a escovação, associado ou não aos produtos formados no esmalte pelo tratamento com os dentifrícios. Em ambos os estudos, foi empregado um delineamento cruzado e duplo cego. No estudo 1, amostras de saliva não estimulada de 5 voluntários foram coletadas antes e imediatamente após a escovação e nos tempos 1, 2, 3, 4, 5, 10, 15, 20, 30, 45 e 60 min após a escovação com BC ou CC, seguida ou não de enxágüe. A área sob a curva da concentração de F na saliva versus tempo foi calculada para determinar a biodisponibilidade de F salivar. Esta foi reduzida em 2,5 x pelo enxágüe pós-escovação ($p<0,05$) e foi semelhante quando BC foi utilizado sem enxágüe e CC foi seguido de enxágüe ($p>0,05$). No estudo 2, doze voluntários realizaram escovação com dentifrícios contendo concentrações de F distintas (placebo (P) – controle negativo, CC ou BC) e utilizaram um dispositivo palatino contendo blocos de esmalte bovino, previamente tratados ou não com suspensão do respectivo dentifrício. Os blocos foram cobertos com uma placa teste de *S. mutans* IB 1600 e após 30 min *in situ*, a placa foi coletada e a concentração de F no fluido foi determinada através de técnica microanalítica com eletrodo íon específico. Um bochecho com sacarose foi realizado como desafio cariogênico e após 45 min os blocos remanescentes e a placa teste foram coletados para avaliação, respectivamente, da perda mineral (simulando o efeito de diferentes espessuras de placa) e da concentração de F no fluido. O pré-tratamento dos blocos de

esmalte com os dentifrícios fluoretados isoladamente não impediu a perda mineral em relação ao controle ($p>0,05$), mas causou aumento na concentração de F no fluido da placa ($p<0,05$). A escovação com os dentifrícios fluoretados aumentou a concentração de F no fluido da placa, sendo encontrada diferença significativa entre BC e CC ($p<0,05$), além de uma menor perda mineral em relação ao controle ($p<0,05$). Adicionalmente, embora a perda mineral tenha sido semelhante para BC e CC na simulação de espessura de placa de até 0,5 mm, ela foi maior para BC na placa mais espessa (1 a 1,5 mm) ($p<0,05$). Os resultados sugerem que o dentifrício de concentração convencional é mais efetivo do que o de baixa concentração na inibição da perda mineral. Adicionalmente, deve-se estimular o enxágüe da boca após o uso do dentifrício de concentração convencional por crianças de pequena idade.

Palavras-chave: Dentifrício, Fluoreto, Fluido da Placa, Desmineralização, Baixa Concentração.

ABSTRACT

The anticaries efficiency of fluoride (F) dentifrices containing 1000-1500 µg F/g is well established, but they are considered a risk factor to dental fluorosis. In order to reduce this risk, low-F concentration dentifrices (500-550 µg F/g) have been recommended, but their anticaries efficiency has not been demonstrated. Thus, this study aimed to: 1. compare salivary F availability after brushing with low-F concentration (LC, 500 µg F/g, NaF) or conventional F concentration (CC, 1100 µg F/g, NaF) dentifrices, followed or not by a water rinse and 2: evaluate *in situ* the anticaries potential of these dentifrices, studying the anticaries effect of F available on the dental biofilm after brushing, associated or not to F products formed on enamel by F dentifrice application was evaluated. In both studies, a crossover, double blind design was used. In study 1, samples of non-stimulated saliva from 5 volunteers were collected before and immediately after brushing with LC or CC, followed or not by a rinse, and after 1, 2, 3, 4, 5, 10, 15, 20, 30, 45 and 60 min. The area under the curve of salivary F concentration versus time was calculated to determine F bioavailability in saliva. F salivary bioavailability was reduced 2.5 X by the post-brushing rinse ($p<0.05$) and it was similar when LC was used without rinsing and CC was used followed by a rinse ($p>0.05$). In study 2, twelve volunteers brushed with dentifrices containing distinct F concentrations (placebo (P) – negative control, LC or CC) and used a palatal appliance containing bovine enamel blocks previously treated or not with a slurry of assigned dentifrice. The blocks were covered with a test plaque from *S. mutans* IB 1600 and after 30 min *in situ*, F concentration in the fluid of plaque was assessed. A sucrose rinse was performed as a cariogenic challenge and after 45 min the remaining blocks and plaque test were removed to evaluate, respectively, mineral loss (as a function of plaque thickness) and F concentration in plaque fluid. The isolated effect of the pretreatment of enamel blocks with F dentifrices did not reduced mineral loss when compared to the control ($p>0.05$), but resulted in higher F concentration in the plaque fluid ($p<0.05$). Brushing with F dentifrices increased F concentration in the plaque fluid, which was significantly different between LC and CC ($p<0.05$), and

resulted in lower mineral loss when compared to the control ($p<0.05$). Additionally, although LC and CC did not differ when mineral loss was evaluated on a plaque thickness simulation of up to 0.5 mm, CC was more efficient than LC at thicker plaque (1 to 1.5 mm) ($p<0.05$). The results suggest that conventional F concentration dentifrice is more efficient than the low-F one in the inhibition of mineral loss. Additionally, post-brushing rinse should be recommended after the use of conventional F concentration dentifrices by young children.

Key words: Dentifrice, Fluoride, Plaque fluid, Demineralization, Low Concentration.

SUMÁRIO

INTRODUÇÃO

O uso de dentifrícios fluoretados é considerado a principal razão para a redução dos índices de cárie que foi observada nas últimas décadas do século XX, tanto em países desenvolvidos quanto nos países em desenvolvimento (Rölla *et al*., 1991; Cury *et al*., 2004). A importância desse método de prevenção da cárie baseia-se na desorganização ou remoção do biofilme dental pelo ato mecânico da escovação, associado ao aporte de fluoreto (F) para o meio ambiente bucal (Rölla *et al*., 1991). Este íon age físico-quimicamente reduzindo a desmineralização dental quando o pH do biofilme dental diminui, pois ao mesmo tempo em que a hidroxiapatita do dente se dissolve, há a precipitação de mineral na forma de fluorapatita. Adicionalmente, quando cessa o desafio cariogênico no biofilme ou na ausência deste, o F presente no meio ambiente bucal ativa a remineralização, favorecendo a precipitação de fluorapatita para repor os minerais perdidos (ten Cate, 1999).

A escovação com dentifrício fluoretado aumenta a biodisponibilidade de F na saliva (Brunn *et al*., 1984; Duckworth & Morgan, 1991), e este parâmetro tem sido utilizado para estimar o potencial anticariogênico de dentifrícios fluoretados. Isso ocorre porque a partir da saliva, o F se difunde para o biofilme e seu fluido, onde age nos processos des-remineralização na interface dente-biofilme (Vogel *et al*., 1992; Ekstrand, 1997). Em acréscimo ao aumento da concentração de F na saliva e no biofilme momentaneamente após a escovação com dentifrícios fluoretados (Duckworth & Morgan, 1991), a utilização freqüente propicia a manutenção de níveis de F elevados no biofilme total (Duckworth & Morgan, 1991; Paes Leme *et al*., 2004; Cenci *et al*., 2008) e também no fluido do biofilme (Cenci *et al*., 2008) mesmo cerca de 10 h após a escovação.

Adicionalmente, quando um produto fluoretado (>100 µg F/g) é aplicado sobre o dente, o F reage com o mineral da superfície dental, formando um depósito tipo fluoreto de cálcio ("CaF_2") (ten Cate, 1997, como revisão). Estas partículas de "CaF_2" funcionariam como um reservatório de F no dente, que é

mobilizado durante os ciclos de pH no biofilme dental (Rölla, 1988). Embora não seja conhecido o exato mecanismo envolvido na manutenção de concentrações maiores de F no biofilme pela utilização freqüente de dentifrícios fluoretados, o "CaF_2" formado na superfície dental pela exposição ao dentifrício (Cruz *et al*., 1992) poderia ser uma fonte de F para o biofilme. Embora a formação de "CaF_2" e seu efeito anticárie estejam bem documentados como resultado da aplicação tópica profissional de F (Rölla, 1988; Ögaard *et al*., 1990), sua formação a partir do uso de dentifrícios fluoretados e a importância desse reservatório no efeito anticárie destes não tem sido explorada na literatura.

Em acréscimo à discussão do mecanismo de ação dos dentifrícios fluoretados, surgem questões relacionadas à segurança de seu uso por crianças de pequena idade. Nesse sentido, dentifrícios com baixa concentração de F (500-550 µg F/g) têm sido recomendados para essas crianças como alternativa para reduzir o risco de fluorose dental (Horowitz, 1995). Entretanto, a eficiência dos dentifrícios de baixa concentração de F na prevenção da cárie, quando comparada àquela dos dentifrícios de concentração convencional, contendo 1000-1500 µg F/g (Marinho *et al*., 2007), ainda não foi demonstrada (Ammari *et al*., 2003; Lima *et al*., 2008). Adicionalmente, para crianças com atividade de cárie o dentifrício de baixa concentração de F é significantemente menos efetivo do que o convencional na regressão ou inibição da progressão de lesões de cárie (Lima *et al*., 2008). Assim, informações adicionais sobre o mecanismo de ação dos dentifrícios de baixa concentração de F, em comparação com os convencionais, são importantes na recomendação de uso desses dentifrícios, como por exemplo, estudar a importância relativa do aumento da concentração de F no biofilme dental versus a formação de reservatórios de "CaF_2" no esmalte dental.

Por outro lado, para reduzir o risco de fluorose dental pelo uso do dentifrício de concentração convencional de F, algumas recomendações têm sido feitas, tais como supervisionar a escovação, aplicar na escova pequena quantidade de dentifrício (Levy *et al*., 2000; Paiva *et al*., 2003) e realizar a

escovação em seguida às refeições (Cury *et al*., 2005). Estimular o enxágüe (lavar a boca com água) logo após a escovação também é aconselhável, uma vez que pode diminuir significativamente a quantidade de F ingerido e absorvido (Sjögren *et al*., 1994). Entretanto, dados epidemiológicos têm demonstrado que os hábitos de enxágüe pós-escovação podem afetar de maneira significativa o índice de cárie de crianças de cerca de 12 anos. Isto é, quão mais efetivo for o enxágüe após a escovação, menor será a eficiência anticárie do dentifrício F utilizado (Chesters *et al*., 1992). Esta diminuição na eficiência do dentifrício F está relacionada com uma menor disponibilidade de F na saliva quando um enxágüe mais abundante é realizado após a escovação utilizando um dentifrício de concentração convencional de F (Duckworth *et al*., 1991; Sjögren & Birkhed, 1994), mas a comparação deste efeito de diminuição da eficiência anticárie do dentifrício em função da diminuição da disponibilidade do F salivar é limitada quando o dentifrício de baixa concentração é utilizado (Issa & Toumba, 2004). Como o hábito de enxágüe da cavidade bucal após a escovação dos dentes é muito difundido e interfere significantemente na disponibilidade de F na cavidade bucal, a avaliação da disponibilidade de F na saliva após escovação com dentifrício de baixa concentração ou convencional, seguida ou não de enxágüe, acrescenta informações para a recomendação de uso desses dentifrícios por crianças de pequena idade em termos de riscos-benefícios.

Assim o objetivo deste trabalho foi: 1. comparar a disponibilidade de F na saliva após utilização de dentifrício com concentração convencional ou de baixa concentração de F, frente à realização de um enxágüe pós-escovação; e 2: avaliar *in situ* o efeito anticariogênico desses dentifrícios e mecanismos envolvidos.

CAPÍTULO 1: Low-fluoride dentifrice and the effect of post-brushing rinsing on fluoride availability in saliva

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ABSTRACT

Aim: Post-brushing water rinsing may reduce the risk of fluoride (F) ingestion from dentifrice, however the decreased salivary F bioavailability may compromise any consequent anticaries benefits. As the use of low-F concentration dentifrices is still a matter of debate, a comparison was made of between the salivary F bioavailability after brushing with a conventional F dentifrice followed by a water rinse and after brushing with a low-F dentifrice without post-brushing rinse. **Study design and methods:** In a crossover, blind study, F concentration in saliva of 5 adult volunteers was determined after brushing with a low-F dentifrice (500 µg F/g) or with a conventional F concentration dentifrice (1100 µg F/g), followed or not by a 15-mL water rinse. **Results:** Salivary F bioavailability was reduced by 2.5 times when a water rinse was used ($p<0.05$), irrespective of dentifrice concentration, and it was 2 times lower for the low-F dentifrice ($p<0.05$). The salivary F bioavailability was similar when the low-F dentifrice was used without post-brushing rinse and the conventional F dentifrice was followed by a rinse ($p>0.05$). **Conclusion:** Habits of post-brushing rinse should be taken into account on the recommendation of dentifrice use by young children, considering the risks and benefits balance of F use.

INTRODUCTION

Fluoride (F) dentifrice is considered to be mainly responsible for the decline in dental caries observed in the last decades either in developed [Rölla et al., 1991] or developing countries [Cury et al., 2004]. The anticaries efficacy of conventional F dentifrices containing 1100-1500 µg F/g is well established [Marinho et al., 2003], but they are considered a risk factor to dental fluorosis [Mascarenhas, 2000].

In order to reduce fluorosis risk, low-F dentifrices (500-550 µg F/g) have been recommended as an alternative for young children [Horowitz, 1995]. However

their anticaries efficacy, when compared with the conventional F concentration dentifrice, has not yet been demonstrated [Ammari et al., 2003]. In addition, for caries-active children, a low-F dentifrice is significantly less effective than a conventional toothpaste in controlling the progression of lesions [Lima et al., 2008].

In spite of the use of low-F dentifrice, other recommendations have been made to reduce the risk of fluorosis by the use of a conventional dentifrice, such as toothbrushing supervision, applying a small amount of dentifrice on the toothbrush [Levy et al., 2000; Paiva et al., 2003] and brushing soon after meals [Cury et al., 2005]. In adittion, use of a post-brushing rinse should be advisable, as it can significantly decrease the amount of F ingested and absorbed [Sjögren et al., 1994]. However, epidemiological data have shown that post-brushing rinsing habits can significantly affect the caries outcome in a young population, i.e. the more effective the rinse, the lower the anticaries efficiency of the dentifrice used [Chesters et al., 1992]. This was shown to be related to a lower F availability in saliva when rinsing more thoroughly after toothbrushing using a dentifrice containing the conventional F concentration [Duckworth et al., 1991; Sjögren and Birkhed, 1993, 1994]. But comparison of this effect on salivary F availability when a low-F dentifrice is used is still limited [Issa and Toumba, 2004].

Accordingly a further study was carried out with an aim of contributing more information concerning the recommendations of F dentifrice use for children. The effect of a water rinse after brushing with low-F dentifrice on the salivary F bioavailability was compared with a conventional dentifrice.

METHODS

Experimental Design. The study was blind to examiners and used a crossover design. Five adults brushed their teeth for 1 min with either a low-F concentration dentifrice (500 µg F/g) or a conventional F dentifrice (1100 µg F/g), both as NaF. In one set of experiments, brushing with each dentifrice was followed by a water rinse,

and in the other set, no rinse was performed. Non-stimulated saliva samples were collected immediately before brushing and up to 60 min after brushing. Salivary F concentration was determined immediately using a F electrode in a micro analytical apparatus [Vogel et al., 1997].

The response variables considered were the salivary F concentration at each time, the maximum salivary concentration (Cmax) and the area under the curve (AUC) of salivary F concentration x time (µg F/mL x min^{-1}). For statistical comparisons, each volunteer was considered as an experimental unit.

Volunteers. Five healthy adult volunteers were recruited to participate in the study. They were 20 to 37 years old (mean age 28.6 years), had a normal salivary flow rate and complete natural dentition, with mean DMFS of 11.2.

Dentifrices. The dentifrices used were commonly available in the Brazilian market. The low-F dentifrice (Colgate Baby®, Colgate, São Paulo, Brazil) contained 500 µg F/g as NaF, in a silica base and tutti-frutti flavour, marketed for young children's use. The conventional F dentifrice (Sorriso Fresh®, Colgate, São Paulo, Brazil) contained 1,100 µg F/g as NaF in a silica base and pepper-mint flavour.

Experimental protocol. Using a computer-generated list, volunteers were randomly assigned to start the experiment using one of the dentifrices and rinsing protocols. According to the crossover design, at the end of the experiment all volunteers had participated in all four combinations of dentifrice concentration and rinsing protocols. Volunteers were instructed to avoid F-rich foods and beverages during the experiment, but no recommendation was made with respect to brushing habits and no F-free washout period was allowed. All saliva collection started at least 2 hrs after breakfast, and during the 1-hr collection, volunteers refrained from speaking, eating or drinking. When no water rinse was performed, volunteers were asked to brush for 1 min with 1.0 g of the assigned dentifrice and expectorated the dentifrice foam. When a water rinse was performed, after expectorating the foam, volunteers rinsed their mouth with 15 mL of distilled water for 10 secs. Unstimulated saliva samples were collected before (baseline) and immediately

after brushing/rinsing (time zero) and at times 1, 2, 3, 4, 5, 10, 15, 20, 30, 45 and 60 min after brushing. As the analytical technique used for F analysis requires microliter volumes of samples [Vogel et al., 1997], collection consisted of only one expectoration of the saliva present in the oral cavity at each time point, and no attempt was made to measure the volume of saliva collected.

Determination of F concentration in saliva samples. Saliva samples were clarified by centrifugation for 2 min at 15,000 x g and diluted with TISAB III (Thermo Electron, Waltham, MA, USA) (10 parts of saliva:1 part of TISAB III). Samples were applied, using micropipettes, on the surface of an oil-covered inverted F electrode with the help of a microscope. A micro-reference electrode, held in a micromanipulator, was used to close the circuit and allow the determination of F concentration in each sample, against standards of known F concentration [Vogel et al., 1997]. Measurements were repeated three times and the variation coefficient among them was lower than 3%. The area under the curve (AUC) of salivary F concentration x time (μg F/mL x min^{-1}) was calculated from time zero to 60 min using the program Origin 6.0 (Microcal Software, Inc., Northampton, MA, USA).

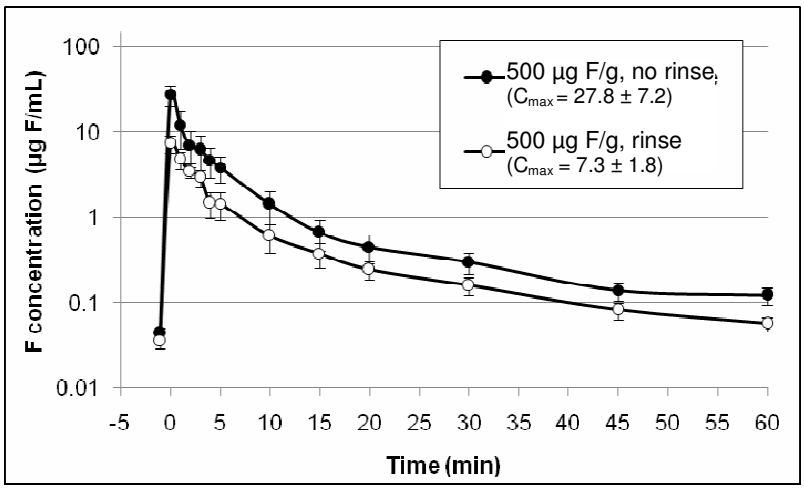
Statistical analysis. The effect of different combinations of dentifrices and rinsing protocols (groups) was tested using ANOVA, considering volunteers as a source of variation. In order to fit the assumptions of normal distribution of errors and equality of variances, data were log transformed. All analyses were performed using the SAS software (SAS Institute Inc., version 8.01, Cary, NC, USA), with p level fixed at 5%.

RESULTS

Figure 1 shows the curves of salivary F concentration x time for the 4 treatment/groups. At baseline (plot -1), F concentration in saliva (mean ± SE) was 0.044 ± 0.007 μg/mL, and no significant difference was found among groups ($p>0.05$).

Immediately after brushing/rinsing, salivary F concentrations significantly increased for all groups ($p<0.001$) (Figure 1), and the maximum values (Cmax) were found immediately after use of the product (time = 0). Cmax values were not significantly different ($p>0.05$) when the same rinse protocol was used, i.e., when the dentifrices were followed or not by a water rinse. Also, no significant difference ($p>0.05$) was observed in the Cmax values between the 500 µg F/g dentifrice used without rinsing and the 1100 µg F/g dentifrice followed by the water rinse. At the 60 min timepoint, the only significant difference observed was between groups 500 µg F/g followed by the rinse and 1100 µg F/g with no rinse. However, for all groups the 60 min salivary F concentration, ranging from 0.06 to 0.23 µg F/mL, was still higher than the baseline values ($p<0.001$) (Figure 1).

The AUC of salivary F concentration x time is shown in Figure 2. The highest F availability was observed by the use of the 1100 µg F/g dentifrice not rinsed, and the lowest by the use of the 500 µg F/g dentifrice followed by a water rinse. No significant difference was found between groups 1,100 µg F/g followed by the rinse and 500 µg F/g used with no rinse ($p>0.05$).



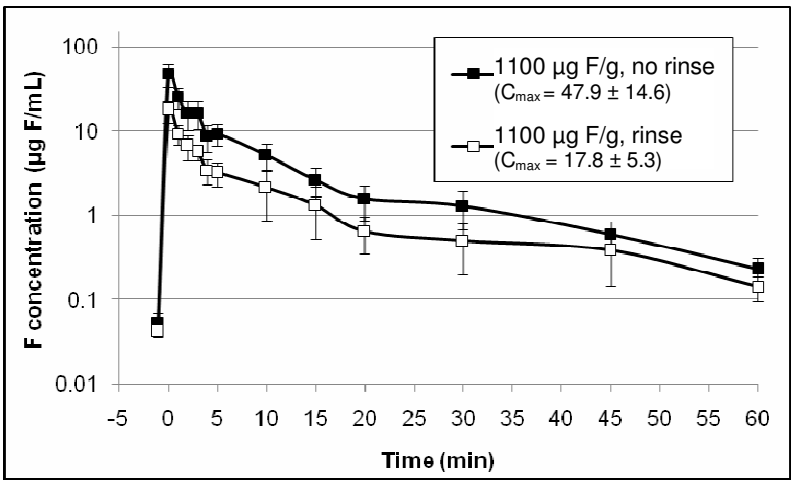


Figure 1. Salivary F concentration (mean ± SE) after the use of 500 or 1100 µg F/g dentifrices, followed or not by a water rinse. Baseline values are plotted at time -1. F concentration values are plotted in log scale. Cmax (mean± SE) are shown at the legend. The statistical significance is described in the Results section.

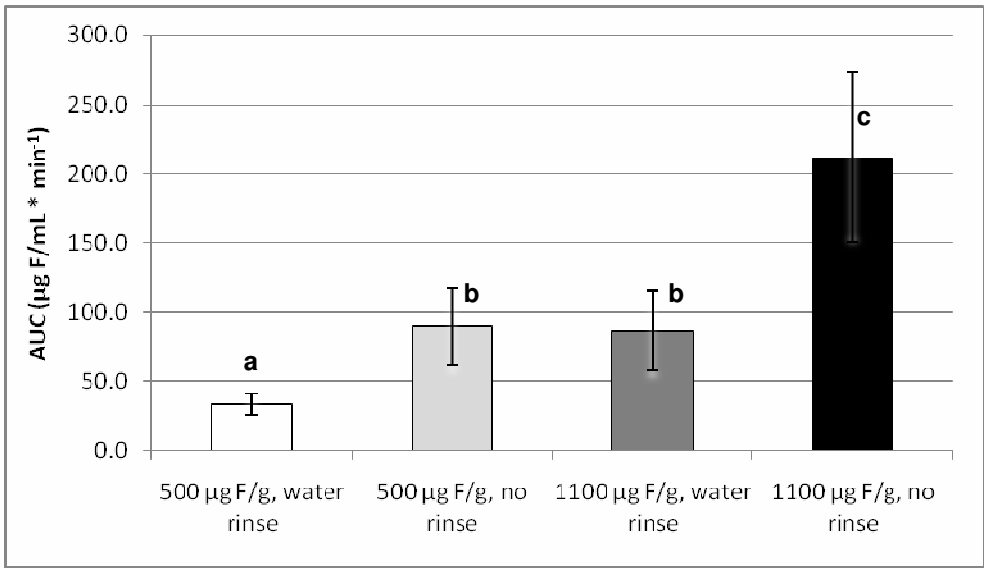


Figure 2. Mean ± SE of the area under curve (AUC) of salivary F concentration x time (µg F/mL x min^{-1}) according to the groups tested. Distinct letters represent groups that differ significantly ($p<0.001$).

DISCUSSION

Salivary F concentration is considered an indicator of F availability to plaque fluid [Vogel et al., 1992; Ekstrand 1997], where the degree of saturation with respect to fluorapatite could decrease the tooth net demineralization when plaque pH drops, and enhance mineral deposition when the pH returns to baseline values [ten Cate, 1999, for review], and consequently can be used to evaluate the anticaries potential of the different dentifrices and the effect of the rinsing protocols tested [Duckworth et al., 1991; Sjögren and Birkhed, 1993, 1994].

The rapid initial decrease in salivary F (Figure 1), which is commonly observed after use of a dentifrice is consistent with a two-compartment open pharmacokinetic model [Duckworth and Morgan, 1991]. Sixty minutes after brushing, F levels in saliva were still higher than baseline (Figure 1), in agreement to other studies [Bruun et al., 1984; Duckworth and Morgan, 1991; Afflitto et al., 1992; Issa and Toumba, 2004], showing that a longer time is necessary for the salivary F concentration to return to the original values.

The effect of rinsing on salivary F was found for both dentifrices in all the parameters evaluated (Figures 1 and 2). Thus, both the Cmax and the AUC values were drastically reduced in 2-4 times by a single water rinse with 15 mL of water, in agreement to others studies [Sjögren and Birkhed, 1994; Issa and Toumba, 2004]. Thus, the anticaries effect of either the conventional dentifrice or the low-F one could be jeopardized by the use of water rinse, and the detrimental effect on the conventional dentifrice has been reported in retrospective and prospective studies [Chesters et al., 1992; Sjögren and Birkhed, 1993; Sjögren et al., 1995]. Considering that there is a dose-response effect between F concentration in dentifrice and caries reduction [Stephen et al., 1988], an important negative effect of water rinse when the low-F dentifrice is used is to be expected. Furthermore, there is no consensus about the anticaries effect of low-F dentifrice [Ammari et al., 2003], and in addition its efficacy may depend on the caries activity of children [Lima et al., 2008]. As rinsing with water could minimize F swallowing after

toothbrushing [Sjögren et al., 1994] and the conventional F dentifrice use would be of a greater benefit to caries-active children [Lima et al., 2008], its use would be more recommendable than the low-F in terms of benefits/risks balance of F use.

There are some limitations of the present study, as the low-F dentifrice used had a child-appealing taste, while the conventional dentifrice had a pepper-mint flavour. However, the difference between the two dentifrices could not be explained by the effect of the different flavours on salivary flow, since the ratio of concentration (1,100/500 = 2.2) was also found in the salivary parameters evaluated (Figures 1 and 2) when the dentifrices were compared in the two conditions. Additionally, this study was conducted with young adults, not children, which could represent a limitation to the findings. Thus, the amount of dentifrice used for brushing was set at 1.0 g, more than twice the pea-size amount recommended for children [Levy et al., 2000; Paiva et al., 2003]. This could partially compensate for the differences in the residual volume of saliva and salivary flow between children and adults [Lagerlöf and Dawes, 1984; Watanabe and Dawes, 1990]. Furthermore, the factors under study were the F concentration in the dentifrice and the effect of rinsing, and as all volunteers were subjected to all treatments, the findings may have significance for children. The crossover design used, considering the volunteers as a source of variation in the statistical analysis, allowed significant differences to be found with the sample size used.

CONCLUSION

Considering that the anticaries benefit of low-F dentifrices is not well established and that water rinse after brushing, a worldwide habit, could contribute to reduce this effect, dentifrice with conventional F concentration should be preferably recommended for children at age-risk for fluorosis rather than the low-F one.

ACKNOWLEDGEMENTS

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REFERENCES

Afflitto J, Schmid R, Esposito A, Toddywala R, Gaffar A. Fluoride availability in human saliva after dentifrice use: correlation with anticaries effects in rats. J Dent Res 1992;71 Spec No:841-845.

Ammari AB, Bloch-Zupan A, Ashley PF. Systematic review of studies comparing anticaries efficacy of children's toothpaste containing 600 ppm of fluoride or less with high fluoride toothapastes of 1000 ppm or above. Caries Res 2003;37:85-92.

Bruun C, Givskov H, Thylstrup A. Whole saliva fluoride after toothbrushing with NaF and MFP dentifrices with different F concentrations. Caries Res 1984;18:282-288.

Chesters RK, Hutington E, Burchell CK, Stephen KW. Effect of oral care habits on caries in adolescents. Caries Res 1992;26:299-304.

Cury JA, Tenuta LM, Ribeiro CC, Paes Leme AF. The importance of fluoride dentifrices to the current dental caries prevalence in Brazil . Braz Dent J 2004;15:167-174.

Cury JA, Del Fiol FS, Tenuta LMA, Rosalen PL. Low-fluoride dentifrice and gastrointestinal fluoride absorption after meals. J Dent Res 2005;84:1133-1137.

Duckworth RM, Knoop DTM, Stephen KW. Effect of mouthrinsing after toothbrushing with a fluoride dentifrice on human salivary fluoride levels. Caries Res 1991;25:287-291.

Duckworth RM, Morgan SN. Oral fluoride retention after use of fluoride dentifrices. Caries Res 1991;25:123-129.

Ekstrand J. Fluoride in plaque fluid and saliva after NaF or MFP rinses. Eur J Oral Sci 1997;105:478-484.

Horowitz HS. Commentary on and recommendations for the proper uses of fluoride. J Public Health Dent 1995;55:57-62.

Issa AI, Toumba KJ. Oral fluoride retention in saliva following toothbrushing with child and adult dentifrices with and without water rinsing. Caries Res 2004 ;38:15-19.

Lagerlöf F, Dawes C. The volume of saliva in the mouth before and after swallowing. J Dent Res 1984;63:1266-1270.

Levy SM, McGrady JA, Bhuridej P, et al. Factors affecting dentifrice use and ingestion among a sample of U.S. preschoolers. Pediatr Dent 2000;22:389-394.

Lima TJ, Ribeiro CCC, Tenuta LMA, Cury JA. Low-fluoride dentifrice and caries lesions control in children with different caries experience: a randomized clinical trial. Caries Res 200**8**,42:46-50.

Marinho VC, Higgins JPT, Logan S, Sheiham A. Fluoride toothpastes for preventing dental caries in children and adolescents. Cochrane Database of Systematic Reviews 2003, Issue 1. Art. No.: CD002278.

Mascarenhas AK. Risk factors for dental fluorosis: a review of the recent literature. Pediatr Dent 2000;22:269-277.

Nagpal DI, Damle SG. Comparison of salivary fluoride levels following use of dentifrices containing different concentrations of fluoride. J Indian Soc Pedod Prev Dent 2007;25:20-22.

Paiva SM, Lima YBO, Cury JA. Fluoride intake by Brazilian children from two communities with fluoridated water. Comm Dent Oral Epid 2003;31:184-191.

Rølla G, Ogaard B, Cruz RA. Clinical effect and mechanism of cariostatic action of fluoride-containing toothpastes: a review. Int Dent J 1991;41:171-174.

Sjögren K, Birkhed D. Factors related to fluoride retention after toothbrushing and possible connection to caries activity. Caries Res 1993;27:474-477.

Sjögren K, Birkhed D. Effect of various post-brushing activities on salivary fluoride concentration after toothbrushing with a sodium fluoride dentifrice. Caries Res 1994;28:127-131.

Sjögren K, Ekstrand J, Birkhed D. Effect of water rinsing after toothbrushing on fluoride ingestion and absorption. Caries Res 1994;28:455-459.

Sjögren K, Birkhed D, Rangmar B. Effect of a modified toothpaste technique on approximal caries in preschool children. Caries Res 1995;29:435-441.

Stephen KW, Creanor SL, Russell JI, et al. A 3-year oral health dose-response study of sodium monofluorophosphate dentifrices with and without zinc citrate: anti-caries results. Comm Dent Oral Epid 1988;16:321-325.

ten Cate JM. Current concepts on the theories of the mechanism of action of fluoride. Acta Odontol Scand 1999;57:325-329.

Vogel GL, Mao Y, Carey CM, Chow LC, Takagi S. In vivo fluoride concentrations measured for two hours after a NaF or a novel two-solution rinse. J Dent Res 1992;71:448-452.

Vogel GL, Mao Y, Carey CM, Chow LC. Increased overnight fluoride concentrations in saliva, plaque, and plaque fluid after a novel two-solution rinse. J Dent Res 1997;76:761-767.

Watanabe S, Dawes C. Salivary flow rates and salivary film thickness in five-year-old children. J Dent Res 1990 ;69:1150-1153.

CAPÍTULO 2: Mechanisms of inhibition of enamel demineralization by conventional and low-fluoride dentifrices

Este Capítulo será submetido ao periódico *Caries Research*

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Short Title: Anticariogenic effects of dentifrices

Key Words: dentifrice, fluoride, plaque fluid, demineralization, low concentration

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Abstract

The anticaries efficiency of fluoride (F) dentifrice is well established, but the relative importance of F diffused to dental plaque remnants during brushing and/or F products formed on cleaned enamel by dentifrice application is unknown. This was evaluated using conventional (FD; 1,100 µg F/g, NaF) and low-F concentration (LFD; 500 µg F/g) dentifrices in a double-blind, crossover, short-term *in situ* study. To evaluate the effect of F in plaque and/or on enamel, 12 volunteers brushed their teeth with no F dentifrice (NF), LFD or FD, and started wearing a palatal appliance containing enamel blocks, pretreated or not with dentifrices, and covered with a test plaque from *S. mutans.* To evaluate the isolated effect of F on enamel, blocks were pretreated with the dentifrices and NF was used for brushing. After 30 min, plaque samples were collected for determination of fluid F concentration, and a rinse with 20% sucrose solution was performed. Demineralization was assessed by change in enamel surface microhardness, simulating plaque thicknesses of 0.05 to 2.5 mm. The pretreatment of enamel blocks with F dentifrices resulted in significantly different F concentrations in plaque fluid ($p<0.05$). However, these values were much lower than those found after brushing with the F dentifrices, and resulted in no significant inhibition of demineralization in comparison to NF ($p>0.05$). A significantly lower demineralization was found when LFD and FD were used for brushing ($p<0.05$), and at a simulated plaque thickness of 1.0-1.5 mm, enamel demineralization was significantly lower for FD ($p<0.05$). The results suggest that F in plaque may be more relevant to inhibit enamel demineralization than products formed on enamel by F dentifrice use, and that conventional F dentifrice (FD) is more effective than the low-F one in reducing enamel demineralization.

Introduction

Fluoride (F) dentifrices are considered to have an important role on dental caries decline observed in the last decades in either developed or developing countries [Rölla et al., 1991; Cury et al., 2004]. Besides plaque disruption, brushing with F dentifrice increases F availability in saliva and plaque leftover after brushing. F available in plaque fluid can interfere with subsequent de- and remineralization events at the tooth-plaque interface [Duckworth and Morgan, 1991; Vogel et al., 1992; Ekstrand, 1997; ten Cate, 1997]. Additionally, F can react with plaque-free enamel or remaining plaque forming F reservoirs (calcium fluoride-like material, CaF_2), which could slowly release F to the plaque fluid during the intervals when dentifrice is not being used [Rölla et al., 1991; ten Cate, 1997].

In fact, in addition to the significant increase in F availability in the mouth immediately after each brushing [Duckworth and Morgan, 1991], the use of F dentifrices is shown to maintain increased F levels in the whole plaque [Duckworth and Morgan, 1991; Paes Leme et al., 2004; Cenci et al., 2008] and in the fluid [Cenci et al., 2008] even 10 h or more after brushing. Although the nature of reservoirs responsible for the maintenance of increased F concentration in plaque is unknown, it could be in part the result of slow release of F reservoirs formed during brushing. However, the importance of F products formed on enamel, as a source of F to the fluid of plaque formed, to inhibit enamel demineralization at the intervals of dentifrice use is unknown.

Also, there is evidence that the anticaries effect of F dentifrices is dependent on their F concentration, i.e. there is a dose-response effect to F concentration in dentifrices [Chesters et al., 1992; ten Cate et al., 2006]. However, the caries preventive efficacy of low-F dentifrices (500-550 µg F/g), recommended to reduce fluorosis risk in young children [Horowitz, 1995], when compared to the well established efficacy of 1,000-1,500 µg F/g dentifrices [Marinho et al., 2006], is still under debate [Ammari et al., 2003; Lima et al., 2008].

Thus, in the present study an *in situ* model was used to evaluate the effect of: 1. F increase in plaque remnants due to brushing; 2. F on enamel due to dentifrice application; and 1 and 2 combined, on the inhibition of enamel demineralization promoted by conventional and low-F concentration dentifrices.

Materials and Methods

Experimental Design

This was a crossover, double blind, split-mouth, short-term *in situ* study, approved by the Research and Ethics Committee of Faculty of Dentistry of Piracicaba (Protocol No. 007/2006). Twelve adult volunteers signed a written, informed consent to participate. They used, in four distinct experimental phases, a palatal appliance containing eight bovine enamel blocks, with predetermined surface microhardness (SMH), in contact with a test plaque prepared from *S. mutans* IB 1600 [Zero et al., 1992; Cury et al., 2003]. The anticaries potential of dentifrices with distinct F concentrations (A. non-F, placebo dentifrice - negative control; B. 500 µg F/g dentifrice; and C. 1,100 µg F/g dentifrice) was evaluated on the inhibition of enamel demineralization after a cariogenic challenge. Two distinct anti-caries mechanisms were evaluated: 1. the effect of F products formed on enamel by dentifrice use and/or 2. F available in plaque after brushing with the dentifrices. To test for the isolated effect of F products formed on enamel by the dentifrices, volunteers used the appliance containing eight enamel blocks, each four previously treated with a slurry of the 500 µg F/g dentifrice or the 1,100 µg F/g dentifrice, and brushed their teeth with the non-F dentifrice. To evaluate the anti-caries effect of F increase in saliva after brushing with the dentifrices, associated or not with F products formed on enamel, volunteers brushed their teeth with the assigned dentifrice and immediately inserted into the oral cavity the palatal appliance containing 4 blocks previously treated with slurry of designed dentifrice and 4 non-treated blocks. In all phases, F in plaque fluid was measured immediately before a cariogenic challenge by a rinse with 20% sucrose solution.

Forty-five min after the cariogenic challenge, the *in situ* test was finished and the anticaries effect of the dentifrices was determined by the mineral loss of enamel blocks and test plaque was also collected for F analysis in the fluid (Figure 1).

Enamel Blocks, Baseline SMH Determination and Treatment with Dentifrices

Enamel blocks (5 X 5 X 2 mm) obtained from bovine incisors were polished plane [Cury et al., 1997, 2000] and their SMH was determined with a Shimadzu HMV-2000 microhardness tester with a knoop indenter using a 50 g load for 5 s. For each enamel block, 11 indentations were made at increasing distances from one block edge (50; 75; 100; 200; 300; 400; 500; 1,000; 1,500; 2,000 and 2,500 µm), which was marked for future reference. The average hardness of these 11 indentations was calculated and a total of 384 blocks presenting a mean hardness of 340.5 kg/mm^2 (SD=30.3) were randomly divided into the treatment/groups.

Blocks used to evaluate the effect of F products on enamel were immersed on a 1:3 (w/w) slurry of the assigned dentifrice for 5 min under agitation (80 rpm) then gently washed for 10 seconds with distilled water and dried with soft tissue paper. This treatment was performed immediately before mounting the enamel blocks in the *in situ* appliance.

Palatal Appliance Mounting

Test plaque was prepared from *S. mutans* Ingbritt-1600 (kindly donated by the Eastman Department of Dentistry, Rochester, NY, USA), as described by Cury et al. [2003]. Palatal appliances carrying two plastic holders were constructed for each volunteer. Four enamel blocks were mounted in each holder, with enamel surfaces in contact with the test plaque. The plastic holders were mounted with the marked edge of the enamel blocks, where the baseline hardness measurements were made, facing the center of the palatal appliance. Further details can be found in Cury et al. [2003].

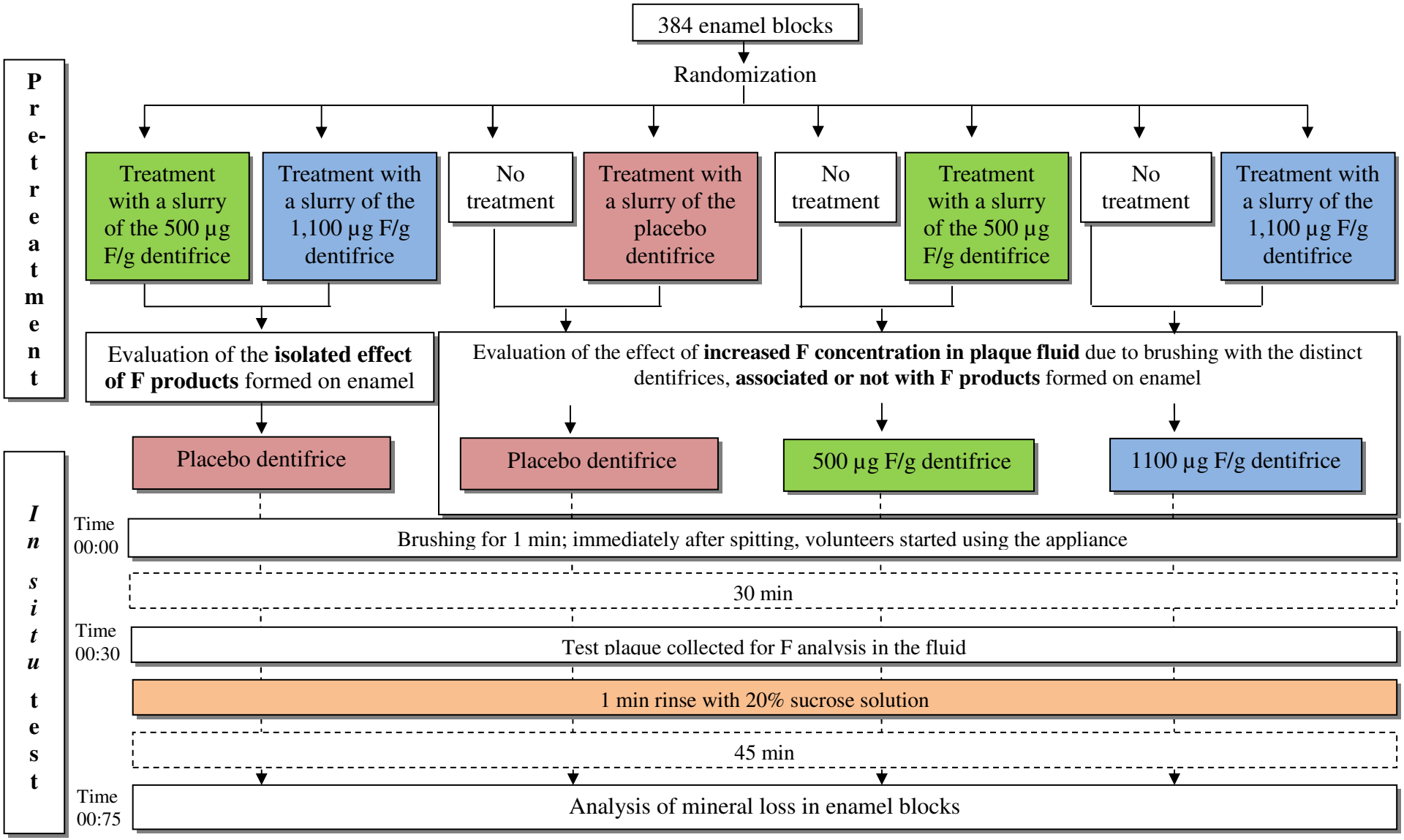

Figure 1: Experimental design of the study.

Intraoral test

Volunteers brushed their teeth with 1.0 g of assigned dentifrice for 1 minute, and immediately after spitting out the dentifrice foam (without rinsing), the appliance was inserted into the mouth. After 30 min, immediately before the cariogenic challenge, two enamel blocks in each holder were removed and test plaque collected for analysis of F concentration in the fluid. A cariogenic challenge was then conducted with the appliance *in situ*, by gently rinsing with 15 mL of 20% sucrose solution during 1 min. Forty-five min after the rinse, the appliance was removed and SMH of the enamel blocks again determined to calculate mineral loss. Test plaque was collected for analysis of F in the fluid and for the change in SMH. During the intraoral test, subjects refrained from talking, drinking or eating.

Collection of plaque and Analysis of F in the Fluid

Test plaque samples were collected using a sterile plastic spatula, and immediately placed inside an oil-filled centrifuge tube [Vogel et al., 1997]. The tube was centrifuged for 5 min (21,000 g) at 4^{o}C to separate the fluid from the plaque solids. The fluid was recovered with oil-filled capillary micropipettes, and immediately analyzed for F concentration, using an inverted F electrode [Vogel et al., 1997]. Samples were applied on the surface of the oil-covered F electrode and diluted with TISAB III (1:10) under microscope. A micro-reference electrode was used to close the circuit, and the signal was read using a high-impedance electrometer (WPI, FD223, Sarasota, FL) coupled to the computer software Plot 1 (Paffenbarger Research Center, ADA Foundation, Gaithersburg, MD).

Analysis of Mineral Loss by Surface Microhardness

Enamel blocks removed from the holders were washed with deionized water and SMH was again measured, at the same distances from the block edge, but at 100 µm from the initial indentations. From this block edge, F remaining in saliva after brushing with the dentifrices and the sucrose solution

diffused through the test plaque, over the enamel surface, simulating a dental plaque thickness of up to 2.5 mm [Zero, 1995]. The percentage of surface microhardness change (%SMC) [%SMC = (SMH after *in situ* test – baseline SMH) x 100/ baseline SMH] was calculated at each distance from the block edge.

Statistical Analysis

Volunteers were considered as statistical blocks. Analysis of variance was used to test for the effect of the dentifrices. In the analysis of concomitant effect of salivary F and F products formed on enamel, the pre-treatment of the blocks was used as a subplot in a split-plot analysis. For the variable %SMC, the distinct distances evaluated were also considered as subplots. Data which did not fit the assumptions of normal distribution of errors and equality of variances were transformed according to Box et al., 1978. SAS software (version 8.01, SAS Institute Inc., Cary, NC, USA) was used and the significance limit was set at 5%.

Results

Isolated Effect of F Products Formed on Enamel by the Dentifrices

Plaque fluid collected before the cariogenic challenge (after 30 min *in situ)* from enamel blocks pre-treated with both F dentifrices had significantly higher F concentrations when compared to the placebo ($p<0.05$, Table 1). Also, plaque fluid F concentration was higher on enamel blocks pre-treated with the 1,100 µg F/g dentifrice when compared to the 500 µg F/g dentifrice ($p<0.05$). Forty-five min after the cariogenic challenge, F concentration in plaque fluid was still higher over blocks pre-treated with both F dentifrices when compared to the placebo ($p<0.05$), but no significant difference was observed between them ($p>0.05$, Table 1).

Table 1: F concentration in plaque fluid (mean ± SD) according to the dentifrice used for pretreatment of the enamel blocks, when the placebo dentifrice was used for brushing.

	F in plaque fluid (µM)	
Pre-treatment of enamel blocks	**30 min after dentifrice use, at the moment of the cariogenic challenge ***	**75 min after dentifrice use (45 min after the challenge) §**
Placebo	2.1 ± 0.6	1.1 ± 0.4
500 µg F/g	9.6 ± 3.1	3.7 ± 2.6
1,100 µg F/g	17.2 ± 4.4	4.6 ± 1.8

* All dentifrices are statistically different from each other ($p<0.05$).
§ Placebo dentifrice is statistically different from both F dentifrices ($p<0.05$). No significant difference was found between the 500 and the 1,100 µg F/g dentifrices ($p>0.05$).

Figure 2 shows the enamel demineralization at the distances from the enamel block edge evaluated. No significant differences in enamel demineralization were observed among the three tested dentifrices ($p>0.05$). However, a significant effect of plaque thickness was observed, with a lower demineralization at 2,000 and 2,500 µm from the block edge ($p<0.05$).

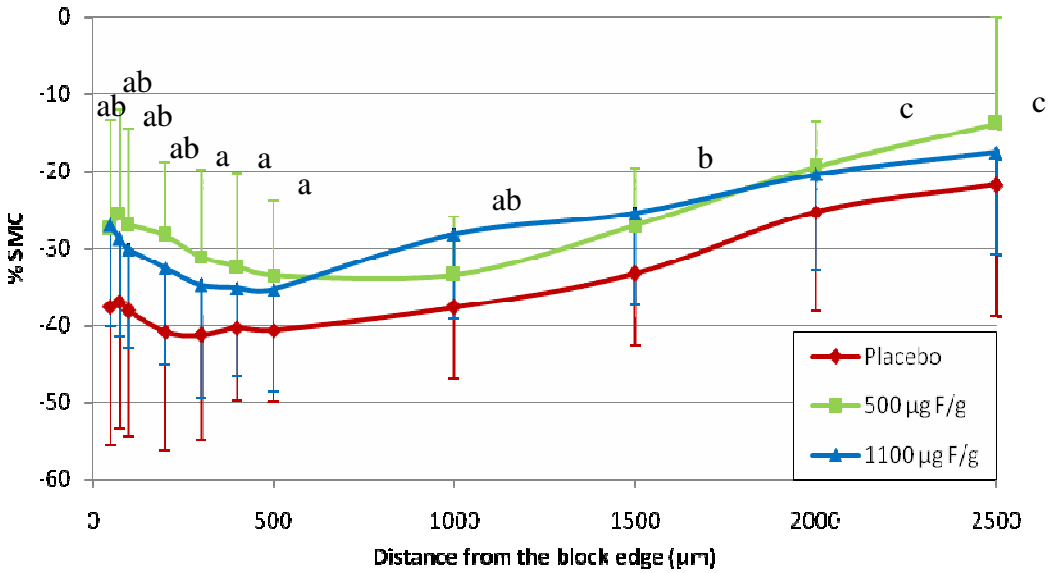

Figure 2: Mean and SD of mineral loss (% surface microhardness change), according to the dentifrice used for pretreating enamel blocks used in the *in situ* demineralization test. Placebo dentifrice was used for brushing at the beginning of the test. No

significant difference was found among dentifrices ($p>0.05$). Letters represent the differences among the distinct distances evaluated ($p<0.05$).

Effect of F in Plaque After Dentifrice Use, Associated or Not with F Products on Enamel

Brushing with the different dentifrices at the beginning of the *in situ* test produced distinct levels of F concentration in the fluid collected 30 min later, the highest for the 1,100 µg F/g dentifrice and the lowest for the placebo dentifrice ($p<0.05$, Table 2). Additionally, no significant effect of the pre-treatment of the enamel blocks was observed for F concentration in the fluid when brushing with the dentifrice was performed 30 min before ($p>0.05$, Table 2).

Table 2: F concentration in plaque fluid (mean ± SD) according to the dentifrice used for brushing at the beginning of the *in situ* test and the pretreatment of the enamel blocks.

		F in plaque fluid (µM)	
Dentifrice	**Pre-treatment of enamel blocks**	**30 min after dentifrice use, at the moment of the cariogenic challenge ***	**75 min after dentifrice use (45 min after the challenge) §**
Placebo	No	2.0 ± 0.4	0.9 ± 0.5
	Yes	2.1 ± 0.6	1.1 ± 0.4
500 µg F/g	No	244.6 ± 290.2	22.9 ± 36.1
	Yes	202.8 ± 222.3	28.4 ± 39.1
1,100 µg F/g	No	508.0 ± 436.5	60.2 ± 74.0
	Yes	524.9 ± 407.6	75.4 ± 67.1

* A statistically significant effect was found for dentifrices (all different from each other, $p<0.05$), but not for the pretreatment of enamel blocks ($p>0.05$).
§ The effects of dentifrices (all different from each other) and pretreatment of blocks (treated different from not-treated) were statistically significant ($p<0.05$).

F concentration in the fluid 45 min after the cariogenic challenge was still significantly different among the 3 dentifrices used for brushing ($p<0.05$, Table 2). At this time, a significant effect of the pre-treatment of enamel blocks

was also observed, with a higher F concentration in the plaque fluid over enamel blocks that were pre-treated with the dentifrice ($p<0.05$, Table 2).

No significant effect of pre-treatment of the enamel blocks was observed in the enamel demineralization ($p>0.05$). Thus, data of not-treated and treated enamel blocks were combined in Figure 3 for clarification. A significant statistical interaction was observed between the factors dentifrice and distance from the block edge ($p<0.05$). For both F dentifrices, the distance from the block edge did not affect enamel demineralization, but for the placebo dentifrice a lower demineralization was found at 2,000 and 2,500 µm from the block edge ($p<0.05$), without significant differences among the other distances ($p>0.05$). At up to 500 µm from the block edge, no significant difference was observed between both F dentifrices ($p>0.05$), which caused a lower demineralization when compared to brushing with the placebo dentifrice ($p<0.05$). However, at 1,000 and 1,500 µm from the block edge, the lowest demineralization was observed when the 1,100 µg F/g dentifrice was used for brushing and the highest when the placebo dentifrice was used. Also, at 2,500 µm, there was no significant difference in enamel demineralization in blocks treated with the placebo or the 500 µg F/g dentifrices, but the 1,100 µg F/g dentifrice presented the lowest demineralization.

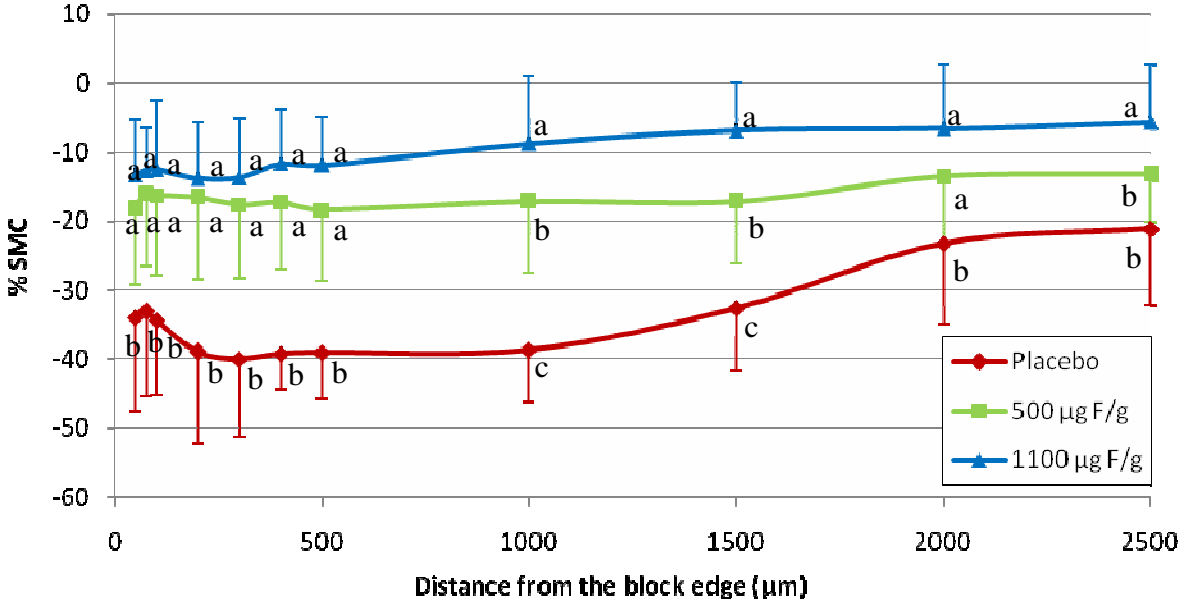

Figure 3: Mean and SD of mineral loss (% surface microhardness change) in enamel blocks used in the *in situ* demineralization test, according to the dentifrice used for brushing at the beginning of the test. Since no significant effect of pretreatment of

enamel blocks was observed, data of treated or not-treated blocks were combined. Distinct letters represent differences among dentifrices at each distance evaluated ($p<0.05$). No significant effect of distance from the block edge was observed for the 500 and 1,100 µg F/g dentifrices ($p>0.05$); but the demineralization at 2,000 and 2,500 µm was significantly lower than block edge, when the placebo dentifrice is considered ($p<0.05$).

Discussion

The experimental model chosen for the present study allowed the distinction of different sources of F to plaque fluid by dentifrice use, i.e. F entering the plaque fluid through saliva, F formed on enamel by dentifrice application, or both, and their importance on the inhibition of enamel demineralization. Interestingly, pretreatment of enamel blocks for 5 min with a slurry of the F dentifrices tested resulted in higher F levels in plaque fluid when compared to the placebo dentifrice used (Table 1), and with significant difference between the different concentration dentifrices. F would come from CaF_2 formed during dentifrice application [Rolla et al., 1991; Cruz et al., 1992], and in fact distinct levels of CaF_2 were found on enamel blocks pretreated with the F dentifrices (data not shown). Although the formation of CaF_2 within clinically relevant exposure times has been questioned [ten Cate, 1997; Harding et al., 1994; Petzold, 2001], our results agrees with those of Bruun and Givskov [1993], who found increased KOH-soluble F values in enamel treated with a slurry of NaF dentifrice for 2 min. Although the amount of CaF_2 formed on enamel surface was small, it resulted in increased F concentrations in the fluid of plaque over it. Thus, F reservoirs formed on enamel by dentifrice use could, at least in part, account for the increased level of F found in whole plaque [Duckworth and Morgan, 1991; Paes Leme et al., 2004; Cenci et al., 2008] and in the fluid [Cenci et al., 2008] by use of F dentifrice.

Although a distinct F level was found in plaque fluid over F dentifrices-treated enamel blocks, this was not able to significantly inhibit enamel demineralization after the sucrose rinse when compared to the placebo dentifrice (Figure 2). This result suggests that F on enamel would not be an important F source once plaque is exposed to a cariogenic challenge. However, in the present study only one exposure to dentifrice was simulated, and alkali-soluble F deposition was shown to increase by daily rinses with sodium fluoride

[Saxegaard and Rölla, 1989]. Thus, the inhibition of mineral loss cannot be overruled considering higher frequencies of dentifrice use. Also, the deposition of CaF_2 by F dentifrices was shown to be higher in caries-like lesions than on sound enamel [Bruun and Givskov, 1993], and consequently would be relevant to enhance enamel remineralization, but this effect was not assessed in the present study.

Also, at the end of the experiment, F in plaque fluid was still higher on enamel blocks treated with both F dentifrices when compared to the placebo (Table 1), but at lower levels when compared to the F concentration before the cariogenic challenge. This suggests that the reservoirs formed by one dentifrice application are leveling off after 75 min in the mouth, but this could have been accelerated by the low-pH environment created by sugar exposure [Rölla and Ögaard, 1986].

The significant effect of F dentifrices on decreasing enamel demineralization is evident when the dentifrices were used for brushing (Figure 3). The higher enamel demineralization after the use of the placebo dentifrice is in agreement with the high cariogenicity of the model used, which allow the diffusion of sucrose through the test plaque and a change in surface microhardness of 40% in the first depths of sugar penetration and around 20% deeper (also seen for the F-treated blocks under placebo dentifrice use, Figure 2). However, after brushing the both F dentifrices, enamel demineralization was significantly decreased when compared to the placebo, and no significant effect of sugar diffusion changing enamel demineralization as a function of plaque thickness was observed. Also, the results demonstrate a higher anticaries effect for the conventional F concentration dentifrice; although demineralization after use of both F dentifrices was not different in the first thicknesses of test plaque evaluated, beginning at 1 mm, the low-F dentifrice was significantly less effective than the conventional F dentifrice. This result suggests that, for thinner plaques both dentifrices would act similarly, but the effect of the low F dentifrice is impaired at thicker plaques, and agrees with recent findings showing that the effectiveness of low-F dentifrices may rely on the caries activity of the study population [Lima et al., 2008].

In fact, although the test plaque was not collected as a function of thickness, a clear dose-response is observed in plaque fluid F concentration after brushing with both F dentifrices (Table 2). Thus the availability of F in plaque after use of conventional F dentifrice was twice higher than after the use of the low-F dentifrice, which could cause the observed effect on enamel demineralization. Also, the high level of F in plaque fluid after brushing with F dentifrices did not allow the observation of any concomitant effect of F products formed on enamel on this increase, or on the inhibition of enamel demineralization.

In conclusion, the results suggest that F products formed on enamel by dentifrice application can increase F concentration in the fluid of plaque formed onto it, but at a lower degree and with minimal effect on enamel demineralization when compared to the burst increase in plaque fluid F after brushing with F dentifrices. Also, the higher F availability in plaque fluid after use of conventional F concentration dentifrices, and the lower demineralization at a simulated deeper plaque when compared to low-F dentifrices, suggest that the latter should be prescribed with caution.

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References

Ammari AB, Bloch-Zupan A, Ashley PF. Systematic review of studies comparing anti-caries efficacy of children's toothpaste containing 600 ppm of fluoride or less with high fluoride toothpastes of 1000 ppm or above. **Caries Res.,** 2003;37:85-92.

Box GEP, Hunter WG, Hunter JS (1978). **Statistics for experimenters**. New York: John Wiley & Sons Inc.

Bruun C, Givskov H. Calcium fluoride formation in enamel from semi- or low-concentrated F agents *in vitro*. **Caries Res.,** 1993;27:96-99.

Cenci MS, Tenuta LM, Pereira-Cenci T, Del Bel Cury AA, ten Cate JM, Cury JA. Effect of microleakage and fluoride on enamel-dentine demineralisation around restorations. **Caries Res.,** 2008, submitted.

Chesters RK, Hutington E, Burchell CK, Stephen KW. Effect of oral care habits on caries in adolescents. **Caries Res.,** 1992;26:299-304.

Cruz R, Ogaard B, Rolla G. Acquisition of alkali-soluble fluoride by enamel through treatment with NaF-containing toothpastes in vitro **Scand J Dent Res.,** 1992;100:81-7.

Cury JA, Rebello MAB, Del Bel Cury AA. In situ relationship between sucrose exposure and the composition of dental plaque. **Caries Res.,** 1997;31:356–360.

Cury JA, Rebelo MAB, Del Bel Cury AA, Derbyshire MTVC, Tabchoury CPM. Biochemical composition and cariogenicity of dental plaque formed in the presence of sucrose or glucose and fructose. **Caries Res.,** 2000;34:491–497.

Cury JA, Francisco SB, Simões GS, Del Bel Cury AA, Tabchoury CP. Effect of a calcium carbonate-based dentifrice on enamel demineralization *in situ*. **Caries Res.,** 2003 May-Jun;37(3):194-9.

Cury JA, Tenuta LM, Ribeiro CC, Paes Leme AF. The importance of fluoride dentifrices to the current dental caries prevalence in Brazil. **Braz Dent J.,** 2004;15 (3):167-74.

Duckworth RM, Morgan SN. Oral fluoride retention after use of fluoride dentifrices. **Caries Res.,** 1991;25(2):123-9.

Ekstrand J. Fluoride in plaque fluid and saliva after NaF or MFP rinses. **Eur J Oral Sci.,** 1997;105:478-84.

Harding AM, Zero DT, Featherstone JDB, McCormack SM, Shields CP, Proskin HM. Calcium fluoride formation on sound enamel using fluoride solutions with and without lactate. **Caries Res.,** 1994;28:1-8.

Horowitz HS. Commentary on and recommendations for the proper uses of fluoride. **J Public Health Dent.,** 1995;55 (1):57-62.

Lima TJ, Ribeiro CCC, Tenuta LMA, Cury JA. Low-fluoride dentifrice and caries lesions control in children with different caries experience: a randomized clinical trial. **Caries Res.,** 2008,42:46-50.

Marinho VCC, Higgins JPT, Logan S, Sheiham A. Fluoride toothpastes for preventing dental caries in children and adolescents (Cochrane Review). In: **Cochrane Library**, Issue 4, 2007. Oxford: Update Software.

Paes Leme AF, Dalcico R, Tabchoury CPM, Del Bel Cury AA, Rosalen PL, Cury JA. *In situ* effect of frequent sucrose exposure on enamel demineralization and on plaque composition after APF application and F dentifrice use. **J Dent Res.,** 2004;83:71-75.

Petzold M. The influence of different fluoride compounds and treatment conditions on dental enamel: a descriptive in vitro study of the CaF_2 precipitation and microstructure. **Caries Res.,** 2001;35:45-51, Suppl 1.

Rölla G, Ögaard B. Studies on the solubility of calcium fluoride in human saliva. In: Factors relating to demineralisation and remineralisation of the teeth. Leach SA, editor. **Oxford: IRL Press**, pp. 45-50, 1986.

Rölla G, Ögaard B, Cruz RA. Clinical effect and mechanism of cariostatic cation of fluoride-containing toothpastes: a review. **Int Dent J.,** 1991;11:442-447.

Saxegaard E, Rölla G. Kinetics of acquisition and loss of calcium fluoride by enamel in vivo. **Caries Res.,** 1989;23:406-11.

ten Cate JM. Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. **Eur J Oral Sci.,** 1997;105:461-465.

ten Cate JM, Exterkate RA, Buijs MJ. The relative efficacy of fluoride toothpastes assessed with pH cycling. **Caries Res.,** 2006;40:136-41.

Vogel GL, Mao Y, Carey CM, Chow LC, Takagi S. In vivo fluoride concentrations measured for two hours after a NaF or a novel two-solution rinse. **J Dent Res.,** 1992;71:448-52.

Vogel GL, Mao Y, Carey CM, Chow LC: Increased overnight fluoride concentrations in saliva, plaque, and plaque fluid after a novel two solution rinse. **J Dent Res.,** 1997;73:761– 767.

Zero DT, Fu J, Anne KM, Cassata S, McCormack SM, Gwinner LM. An improved intra-oral enamel demineralization test model for the study of dental caries. **J Dent Res.,** 1992 Spec Iss;71:871-8.

Zero DT. *In situ* caries model. **Adv Dent Res.,** 1995 Nov;9(3):214-30; discussion 231-4.

CONSIDERAÇÕES GERAIS

O uso de dentifrícios fluoretados tem sido considerado o principal responsável pela redução dos índices de cárie que ocorreu no mundo nas últimas décadas (Rölla *et al*., 1991; Cury *et al*., 2004). Embora a eficiência anticárie dos dentifrícios com concentração convencional de F esteja bem estabelecida, preocupação recente tem surgido com relação ao risco aumentado de desenvolvimento de fluorose dental por sua ingestão por crianças durante o período de formação dos dentes (Mascarenhas, 2000). Assim, dentifrícios de concentração reduzida de F têm sido recomendados (Horowitz, 1995), mas sua eficiência anticárie não está demonstrada na literatura (Ammari *et al*., 2003). Além disso, dados recentes têm sugerido que eles não são eficientes na redução de lesões ou na inibição do aparecimento de novas lesões em crianças com atividade de cárie (Lima *et al*., 2008). Assim, neste trabalho foram estudados diferentes aspectos relacionados ao efeito anticariogênico dos dentifrícios de baixa concentração de F.

Assim, foi objetivo do estudo comparar a disponibilidade de F na saliva após o uso de dentifrícios de concentração convencional ou reduzida de F e o efeito do enxágüe pós-escovação. Como já descrito na literatura, a realização de um enxágüe pós-escovação diminui significativamente a disponibilidade de F na saliva (Duckworth *et al*., 1991; Sjögren & Birkhed, 1994) e o potencial anticárie do dentifrício (Chesters *et al*., 1992). No entanto, estudos acerca desse efeito quando comparado à utilização do dentifrício de baixa concentração são escassos (Issa & Toumba, 2004). Assim, o estudo demonstrou que a realização de um enxágüe após a utilização do dentifrício de baixa concentração de F resulta na menor disponibilidade de F na saliva, o que pode explicar porque ele não é efetivo em crianças com atividade de cárie (Lima *et al*., 2008). Por outro lado, a biodisponibilidade de F na saliva após a utilização do dentifrício convencional seguido de enxágüe não diferiu em relação ao dentifrício de baixa concentração utilizado sem enxágüe. Considerando que o treinamento no hábito de enxágüe da cavidade bucal é importante desde cedo para crianças, visando limitar a ingestão de dentifrícios,

independentemente da idade, e que a eficiência dos dentifrícios de baixa concentração não está demonstrada, sugere-se que o uso de pequena quantidade de dentifrício de concentração convencional de F, seguido de enxágüe, seja recomendado para crianças de pequena idade.

De fato, os resultados do trabalho 2 sugerem que o dentifrício de concentração convencional é mais efetivo na inibição da desmineralização dental em comparação ao de baixa concentração. O modelo *in situ* utilizado já foi previamente empregado com sucesso na avaliação do potencial anticárie de componentes abrasivos de dentifrícios fluoretados (Cury *et al*., 2003), e no presente estudo se mostrou adequado para avaliar dentifrícios de diferentes concentrações de F. O trabalho mostrou um grande aumento na concentração de F no fluido da placa teste de *S. mutans*, utilizada como modelo de biofilme dental, após a escovação com os dentifrícios, demonstrando a penetração do F remanescente na saliva após a escovação, na placa teste. Embora a coleta da placa teste não tenha sido realizada em função da profundidade a partir da superfície exposta à cavidade bucal, os resultados demonstram que a utilização de dentifrício de concentração convencional fornece uma maior concentração de F para a placa teste em relação ao dentifrício de baixa concentração. O efeito anticariogênico superior do dentifrício de concentração convencional foi demonstrado nas profundidades de 1,0 e 1,5 mm da superfície da placa teste, que simulam a espessura do biofilme dental.

O trabalho também demonstrou a reação do F dos dentifrícios formando produto fluoretado ("CaF_2") na superfície do esmalte. Esse reservatório forneceu F para o fluido da placa teste, sendo a primeira demonstração na literatura desse mecanismo. No entanto, frente ao grande aumento na concentração de F na placa após a escovação com os dentifrícios, a dissolução do "CaF_2", fornecendo F para o fluido da placa teste, não foi suficiente para impedir a perda mineral. No entanto, a deposição desse reservatório pode ser importante quando se considera a freqüência diária de uso do dentifrício, repondo esses reservatórios a cada escovação e garantindo a manutenção de níveis ligeiramente elevados de F no fluido do biofilme dental.

CONCLUSÃO

Os resultados sugerem que o aumento na disponibilidade de F no biofilme após a escovação é o principal responsável pelo efeito anticariogênico de dentifrícios fluoretados, sendo que os dentifrícios de baixa concentração resultam em cerca de metade da disponibilidade de F na saliva ou no fluido do biofilme em relação aos de concentração convencional, mesmo quando eles são utilizados seguidos por um enxágüe. Considerando que o efeito anticárie do dentifrício de baixa concentração de F parece ser reduzido, recomenda-se a utilização do dentifrício de concentração convencional, incentivando-se a realização de enxágüe após seu uso.

REFERÊNCIAS*

Ammari AB, Bloch-Zupan A, Ashley PF. Systematic review of studies comparing anti-caries efficacy of children's toothpaste containing 600 ppm of fluoride or less with high fluoride toothpastes of 1000 ppm or above. Caries Res. 2003; 37: 85-92.

Bruun C, Givskov H, Thylstrup A. Whole saliva fluoride after toothbrushing with NaF and MFP dentifrices with different F concentrations. Caries Res. 1984; 18: 282-8.

Cenci MS, Tenuta LM, Pereira-Cenci T, Del Bel Cury AA, ten Cate JM, Cury JA. Effect of microleakage and fluoride on enamel-dentine demineralisation around restorations. Caries Res. submetido 2008.

Chesters RK, Hutington E, Burchell CK, Stephen KW. Effect of oral care habits on caries in adolescents. Caries Res. 1992; 26: 299-304.

Cruz R, Ogaard B, Rolla G. Acquisition of alkali-soluble fluoride by enamel through treatment with NaF-containing toothpastes *in vitro*. Scand J Dent Res. 1992; 100: 81-7.

Cury JA, Francisco SB, Simões GS, Del Bel Cury AA, Tabchoury CPM. Effect of a calcium carbonate-based dentifrice on enamel demineralization *in situ*. Caries Res. 2003; 37: 194-9.

Cury JA, Tenuta LM, Ribeiro CC, Paes Leme AF. The importance of fluoride dentifrices to the current dental caries prevalence in Brazil. Braz Dent J. 2004; 15: 167-74.

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Cury JA, Del Fiol FS, Tenuta LMA, Rosalen PL. Low-fluoride dentifrice and gastrointestinal fluoride absorption after meals. J Dent Res. 2005; 84: 1133-7.

Duckworth RM, Morgan SN. Oral fluoride retention after use of fluoride dentifrices. Caries Res., 1991; 25(2): 123-9.

Duckworth RM, Knoop DTM, Stephen KW. Effect of mouthrinsing after toothbrushing with a fluoride dentifrice on human salivary fluoride levels. Caries Res. 1991; 25: 287-91.

Ekstrand J. Fluoride in plaque fluid and saliva after NaF or MFP rinses. Eur J Oral Sci. 1997; 105: 478-84.

Horowitz HS. Commentary on and recommendations for the proper uses of fluoride. J Public Health Dent. 1995; 55(1): 57-62.

Issa AI, Toumba KJ. Oral fluoride retention in saliva following toothbrushing with child and adult dentifrices with and without water rinsing. Caries Res. 2004; 38: 15-9.

Levy SM, McGrady JA, Bhuridej P, Warren JJ, Heilman JR, Wefel JS. Factors affecting dentifrice use and ingestion among a sample of U.S. preschoolers. Pediatr Dent. 2000; 22: 389-94.

Lima TJ, Ribeiro CCC, Tenuta LMA, Cury JA. Low-fluoride dentifrice and caries lesions control in children with different caries experience: a randomized clinical trial. Caries Res. 2008; 42: 46-50.

Marinho VCC, Higgins JPT, Logan S, Sheiham A. Fluoride toothpastes for preventing dental caries in children and adolescents (Cochrane Review). Cochrane Database Syst Rev. 2007; (4).

Mascarenhas AK. Risk factors for dental fluorosis: a review of the recent literature. Pediatr Dent. 2000; 22: 269-77.

Ögaard B, Rölla G, Ruben J, Arends J. Relative cariostatic effects of KOH-soluble and KOH-insoluble fluoride *in situ*. J Dent Res. 1990; 69: 1505-7.

Paes Leme AF, Dalcico R, Tabchoury CPM, Del Bel Cury AA, Rosalen PL, Cury JA. In situ effect of frequent sucrose exposure on enamel demineralization and on plaque composition after APF application and F dentifrice use. J Dent Res. 2004; 83: 71-5.

Paiva SM, Lima YBO, Cury JA. Fluoride intake by Brazilian children from two communities with fluoridated water. Community Dent Oral Epidemiol. 2003; 31: 184-91.

Rölla G. On the role of calcium fluoride in the cariostatic mechanism of fluoride. Acta Odontol Scand. 1988; 46: 341-5.

Rölla G, Ögaard B, Cruz RA. Clinical effect and mechanism of cariostatic cation of fluoride-containing toothpastes: a review. Int Dent J. 1991; 11: 442-7.

Sjögren K, Birkhed D. Effect of various post-brushing activities on salivary fluoride concentration after toothbrushing with a sodium fluoride dentifrice. Caries Res. 1994; 28: 127-31.

Sjögren K, Ekstrand J, Birkhed D. Effect of water rinsing after toothbrushing on fluoride ingestion and absorption. Caries Res. 1994; 28: 455-9.

ten Cate JM. Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. Eur J Oral Sci. 1997; 105: 461-5.

ten Cate JM. Current concepts on the theories of the mechanism of action of fluoride. Acta Odontol Scand. 1999; 57: 325-9.

Vogel GL, Mao Y, Carey CM, Chow LC, Takagi S. In vivo fluoride concentrations measured for two hours after a NaF or a novel two-solution rinse. J Dent Res. 1992; 71: 448-52.

ANEXO 1: Certificado do Comitê de Ética em Pesquisa

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Disponibilização de íons minerais no fluido de biofilme teste mediante a utilização de dentifrício de baixa concentração de fluoreto - avaliação da inibição da desmineralização e da ativação da remineralização do esmalte**", protocolo nº **007/2006**, dos pesquisadores **LIVIA MARIA ANDALÓ TENUTA, ALTAIR ANTONINHA DEL BEL CURY, CÍNTHIA PEREIRA MACHADO TABCHOURY, CLAUDIA BIANCHI ZAMATARO e JAIME APARECIDO CURY**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 08/02/2006.

Piracicaba, 17 de outubro de 2007

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project "**Availability of mineral ions in the fluid of a test biofilm after the use of a low-fluoride dentifrice - inhibition of enamel demineralization and enhancement of remineralization**", register number **007/2006**, of **LIVIA MARIA ANDALÓ TENUTA, ALTAIR ANTONINHA DEL BEL CURY, CÍNTHIA PEREIRA MACHADO TABCHOURY, CLAUDIA BIANCHI ZAMATARO and JAIME APARECIDO CURY**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 08/02/2006.

Piracicaba, SP, Brazil, October 17 2007

Profa. Cínthia Pereira Machado Tabchoury
Secretária
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

ANEXO 2: Respostas do editor ao artigo 1, submetido no periódico *European Archives of Paediatric Dentistry* em 03/01/2008

Livia Tenuta

De: <Curzongalphay@aol.com>
Para: <litenuta@fop.unicamp.br>
Enviada em: sexta-feira, 29 de fevereiro de 2008 07:41
Anexar: EAPD 07.71.Reviewers comments.doc
Assunto: re EAPD 07.71

Dear Dr Tenuta

I have now received the two scientific reviews of your paper on low-F dentifrice and saliva F. They are attached. Both reviewers feel that the paper can be published but only after a major revision and re-writing of the complete paper. They have made many comments and suggestions which you will need to follow.

When you have read these comments please let me know whether you wish to revise this paper and then re-submit it for consideration. Alternatively you may wish to withdraw the paper.

Thank you for considering the EAPD for your research,

Yours sincerely,

Professor Emeritus Martin.E.J. Curzon.
Editor-in-Chief, European Archives Paediatric Dentistry.
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----- Original Message -----
From: Curzongalphay@aol.com
To: litenuta@fop.unicamp.br
Sent: Monday, March 31, 2008 12:44 PM
Subject: Re: [Fwd: Re: re EAPD 07.71]

Thank you

I will process your paper for publication.

Professor Emeritus Martin.E.J. Curzon.
Editor-in-Chief, European Archives Paediatric Dentistry.
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ANEXO 3: Delineamento experimental do experimento relatado no Capítulo 2