ACTA ODONTOLOGICA SCANDINAVICA, 2017
VOL. 75, NO. 5, 376–378
http://dx.doi.org/10.1080/00016357.2017.1318448

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SHORT COMMUNICATION

Fluoride varnishes containing sodium trimetaphosphate reduce enamel demineralization *in vitro*

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ABSTRACT

Objective: This study evaluated the effects of fluoride varnishes containing sodium trimetaphosphate (TMP) on bovine enamel demineralization *in vitro*.

Material and methods: Enamel bovine discs were randomly assigned into six groups ($n = 20$/group): placebo, 2.5% NaF, 2.5% NaF/5% TMP, 5% NaF, 5% NaF/5% TMP, and a commercial formulation (Duraphat, 5% NaF). Varnishes were applied on all enamel discs and kept for 6 h. Loosely and firmly bound fluoride formed on/in enamel after treatment were analyzed in 10 discs from each group. The other 10 discs were subjected to a pH-cycling regimen for 7 days, and analyzed for surface (SH) and cross-sectional hardness (ΔKHN), as well as for loosely and firmly bound fluoride in/on enamel. Data were analyzed by analysis of variance (ANOVA) followed by Student–Newman–Keuls' test ($p < .05$).

Results: The lowest SH change and ΔKHN were observed for the 5%NaF/5%TMP varnish, which was significantly different from all the other groups. Both fluoridated varnishes containing TMP promoted significantly lower SH change and ΔKHN when compared with their counterparts without TMP. Loosely and firmly bound fluoride was significantly lower in groups treated with varnishes containing TMP.

Conclusion: TMP and fluoride added to varnishes have a synergistic effect against enamel demineralization *in vitro*.

ARTICLE HISTORY
Received 21 February 2017
Revised 30 March 2017
Accepted 8 April 2017

KEYWORDS
Demineralization; dental enamel; fluorine; polyphosphates

Introduction

Considering that fluoride (F) alone is not able to completely prevent, arrest or reverse the caries process, recent attention has been given to alternatives to increase the effectiveness of topically applied fluoridated products. In this sense, the supplementation of F varnishes with sodium trimetaphosphate (TMP) was shown to promote a synergistic effect on the remineralization of caries-like lesions using *in vitro* and *in situ* protocols [1,2]. Since varnishes are not only used as a therapeutic agent for the treatment of white spot lesions, but also recommended for preventing the development of new caries lesions [3], this *in vitro* study assessed the effects of F varnishes containing TMP on enamel demineralization. The null hypothesis was that no additional protective effect would be attained by the addition of TMP to varnishes with different F concentrations.

Material and methods

Varnish formulation and fluoride determination in the products

All varnishes were manufactured by SS White Dental Products (Rio de Janeiro, RJ, Brazil) and contained: colophony, ethyl cellulose, tolu balsam, beeswax, toluene sulfonamide, vanillin, saccharin and ethanol. F concentrations were 2.5 and 5% of NaF (Merck, Darmstadt, Germany), with or without 5% TMP (Sigma-Aldrich Co., St. Louis, MO). A placebo (without F and TMP) and a commercial formulation (Duraphat^{TM}, 5% NaF) were also included. F concentrations in the products were determined with an ion-specific electrode after colophony dissolution by chloroform, as previously reported [4].

Treatment with F varnishes and pH-cycling

Enamel discs (20 discs/group) were obtained from bovine incisors, previously stored in 2% formaldehyde solution pH 7.0 for 30 days [5]. Discs had their surface serially polished using 600, 800 and 1200-grade water-cooled silicon carbide paper disks (Buehler) during 1 min each, with a final polish using a felt disk (Buehler Polishing Cloth 40–7618) moistened with a 1-μm diamond polishing suspension (Extec Corp., Enfield, CT), being subsequently selected by surface hardness (320.0–380.0 KHN). Sample size was based on a previous study with a similar research protocol [1]. Discs were treated with the varnishes and immersed in a demineralizing solution (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/mm^2). After 6 h, varnishes were removed with a blade and acetone and half the discs

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($n = 10$/group) were transferred to 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} cacodylic buffer, pH 7.0; 0.05 mg F/mL, 1.1 mL/mm^2) for 18 h, following the protocol described by Vieira et al. [5]. On the following day, both solutions were replaced by fresh ones due to F release from the varnishes. These solutions were kept until the fifth day. On the sixth and the seventh day, the enamel discs were kept in the remineralizing solution. The other set of specimens (not submitted to the pH-cycling regimen, $n = 10$) was used for the determination of firmly and loosely bound enamel fluoride concentrations formed after application of the varnishes, as described below.

Analysis of enamel hardness

Surface hardness (SH) was determined using a microhardness tester (Buehler, Lake Bluff, IL) and a Knoop diamond under a 25-g load for 10 s [5]. Five indentations spaced 100 μm apart were made at the center of the enamel surface (SH). After pH-cycling, five indentations were made (SH_1), spaced 100 μm apart from each other and from SH, allowing the calculation of the percentage of SH loss (%SH) [5]. For cross-sectional hardness measurements, enamel discs were longitudinally sectioned through their center and embedded in acrylic resin with the cut face exposed and polished. A sequence of 14 indentations at different distances (5, 10, 15, 20, 25, 30, 40, 50, 70, 90, 110, 130, 220 and 330 μm) from the surface of the enamel were created in the central region, under a 5-g load for 10 s [6]. The integrated loss of subsurface hardness (ΔKHN; KHN × μm) was calculated using the hardness values (KHN) and the trapezoidal rule [7].

Fluoride analysis on/in enamel

The amount of loosely bound (CaF_2) and firmly bound F were measured 6 h after varnish application (formed) and 7 days after pH-cycling (retained), in two different sets of specimens ($n = 10$/group). A digital calliper (Mitutoyo CD-15B) was used to measure the surface area of the enamel. Assessment of CaF_2 uptake by enamel was performed according to Caslavska et al. [8]. Enamel biopsy was then performed as reported by Weatherell et al. [9], as modified by Alves et al. [10], for determination of firmly bound F.

Statistical analysis

Analyses were performed using the SigmaPlot software (version 12.0) (SigmaPlot, Systat Software Incorporation, San Jose, CA). The variables %SH, ΔKHN showed normal (Shapiro–Wilk) and homogeneous (Cochran test) distributions and were submitted to one-way analysis of variance (ANOVA) and Student–Newman–Keuls' test. CaF_2 and F data were transformed (cubic root) and subjected to two-way ANOVA followed by Student-Newman-Keuls' test ($p < .05$).

Results

Mean (standard deviation) F concentrations (μg F/g) in the varnishes were 302.2 (62.8), 12,470.2 (712.2), 12,645.5 (603.5), 22,187.5 (1,053.1), 22,061.6 (1,101.5) and 21,107.6 (138.3), respectively for Placebo, 2.5%NaF, 2.5%NaF/5%TMP, 5%NaF, 5%NaF/5%TMP and Duraphat (5% NaF).

A dose-response relationship was observed between F concentration in the varnishes without TMP and %SH and ΔKHN (Table 1). When TMP was used in association with F, significantly lower %SH and ΔKHN were observed in comparison to their counterparts without TMP. Conversely, CaF_2 and firmly bound F concentrations were significantly lower for groups treated with the TMP-containing varnishes.

Discussion

The use of fluoridated varnishes is regarded as a safe, effective and well-accepted measure in the clinical practice, especially by young children due to the prolonged contact time between fluoride and the tooth surfaces, and the possibility of using very small amount of the product [11]. The association between F and TMP in the present study was able to significantly reduce the demineralization of sound enamel using an *in vitro* protocol for both primary outcomes assessed (%SH and ΔKHN), leading to the rejection of the study's null hypothesis. The effects on the subsurface (ΔKHN) are of special interest from a clinical perspective, given that a cavity might take longer to develop when enamel is treated with a TMP-containing varnish in comparison with formulations of same F concentration without TMP.

Similar patterns were observed for surface and cross-sectional hardness, in which the varnishes containing fluoride and TMP significantly reduced enamel demineralization when compared with varnishes containing the same amount of fluoride, without TMP. Such trend had already been described for varnishes in dental caries and erosion using different research protocols [1,2,12,13], as well as for other topically applied fluoridated vehicles. Although the mechanism of action of TMP used in association with fluoride has not already been fully described, this synergistic effect has been attributed to the high affinity of TMP to enamel [14,15].

Unlike traditional fluoridated formulations, in which the anticaries effect has been attributed to the formation of high amount of CaF_2-like material on enamel, as well as to the incorporation of F into deeper layers of enamel (firmly bound fluoride), the addition of TMP to the varnishes significantly reduced the amount of both firmly and loosely bound fluoride when compared with varnishes with same fluoride concentrations without TMP, what in line previous findings on enamel remineralization [1,2]. These studies taken together reinforce the concept that TMP has a strong affinity to enamel [15], leading to a rapid absorption to enamel surfaces, thus acting as a partial barrier to CaF_2 deposition on enamel and to ionic F penetration into enamel. Despite this might be considered as an unwanted result at first glance, the enhanced protective effect of TMP-containing varnishes indicates that such formulations have a different mechanism of action when compared with formulations in which fluoride is the only active ingredient [1].

The results of the present study, however, should be interpreted within the limitations of an *in vitro* protocol.

Table 1. Mean (standard deviation) percentage of enamel surface hardness loss (%SH), integrated subsurface hardness loss (ΔKHN) after pH-cycling, and loosely bound (CaF_2) and firmly bound fluoride (F) concentrations in the enamel 6 h after varnish application (formed) and after pH-cycling (retained).

	Hardness		CaF_2 (μg/cm^2)		F (μg/mm^3)	
Groups	%SH	ΔKHN	Formed^A	Retained^B	Formed^A	Retained^B
Placebo	−73.3^a	5747^a	2.0^a	0.4^a	0.20^a	0.42^a
	(0.5)	(−351)	(0.3)	(0.1)	(0.04)	(0.04)
2.5% NaF	−51.2^b	3931^b	89.4^c	1.8^c	0.53^c	1.16^b
	(2.0)	(253)	(1.8)	(0.3)	(0.07)	(0.11)
5% NaF	−42.5^c	3080^c	166.9^e	4.5^d	0.77^d	2.66^d
	(0.9)	(193)	(4.0)	(0.7)	(0.16)	(0.18)
2.5%NaF/5%TMP	−41.7^c	3003^c	37.9^b	1.2^b	0.39^b	1.09^b
	(0.9)	(92.3)	(0.2)	(0.3)	(0.06)	(0.08)
5%NaF/5%TMP	−25.0^d	2040^d	95.6^c	2.1^c	0.70^d	1.94^c
	(1.2)	(125)	(1.9)	(0.5)	(0.10)	(0.13)
Duraphat	−40.4^c	2992^c	114.5^d	4.7^d	0.74^d	2.08^c
	(1.2)	(187)	(1.7)	(0.8)	(0.11)	(0.15)

Superscript letters indicate differences between CaF_2 or formed and retained for each group (upper case), and among the groups within each column (lower case). Student–Newman–Keuls' test ($p < .05$, $n = 10$).

Therefore, before these formulations can be recommended for clinical use, future studies should be conducted in conditions that better resemble the clinical situation, in which the interactions with salivary pellicle, proteins, buffers and microorganisms are considered, as well as the inclusion of daily oral hygiene procedures (e.g. regular brushing with a fluoridated toothpaste). *In situ* studies would be helpful in that regard, besides providing further evidence for a better understanding of the mechanisms of action of TMP-supplemented fluoridated vehicles.

To conclude, the addition of TMP to fluoride varnishes significantly reduces demineralization of sound enamel *in vitro* when compared to varnishes with same F concentration without TMP, suggesting that this formulation could potentially be used in patients at high caries risk and activity, aiming to prevent the development of new caries lesions.

Acknowledgments

The authors thank CAPES (scholarship to M.M.M. and L.C.B.-Q.) and CNPq (scholarship to F.R.N.M.) for financial support. The authors also thank SS White Dental Products (Rio de Janeiro, Brazil) for the production of the varnishes.

Disclosure statement

A.C.B.D. holds a patent request for a product used in the study, by the National Institute of Industrial Property, INPI/SP, on 04/29/2008, #018080026091, PI0801811-1, published on 11 January 2011.

Funding

The authors thank CAPES (scholarship to M.M.M. and L.C.B.-Q.) and CNPq (scholarship to F.R.N.M.) for financial support.

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