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Comparative *In Vitro* Effect of TiF_4 to NaF and Potassium Oxalate on Reduction of Dentin Hydraulic Conductance

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Clinical Relevance

This study showed that titanium tetrafluoride (TiF_4) varnish and solution were less effective than sodium fluoride (NaF) varnish and potassium oxalate gel in reducing dentin hydraulic conductance. Therefore, TiF_4 might be not successful against dentin hypersensitivity (DH), whereas NaF varnish or potassium oxalate gel could benefit patients with DH.

SUMMARY

Dentin hypersensitivity (DH) is related to an increase in dentin permeability. This study tested the effect of titanium tetrafluoride (TiF_4) compared with sodium fluoride (NaF) and potassium oxalate gel on reducing hydraulic conductance (Lp) from the perspective of diminishing dentin permeability. The Lp of the dentin disks (1.0 ± 0.2 mm) was evaluated using Flodec. The maximum Lp values of each disk were taken after phosphoric acid etching (15 seconds) and randomly allocated to seven groups (n=8) according to the treatments. The minimum (smear layer) and the maximum (after acid etching) Lp values were recorded. Treatments were performed for 4 minutes as follows: 1) NaF varnish 2) and solution (2.45% F, pH 5.0), 3) TiF_4 varnish and 4) solution (2.45% F, pH 1.0), 5) 3% potassium oxalate gel, 6) free fluoride varnish (placebo, pH 5.0), 7) and no treatment (control). The Lp after each treatment was assessed. Samples were exposed to an erosive challenge (6% citric acid, pH 2.1, 1 minute), and the final Lp was recorded. The data were statistically analyzed using repeat-

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ed measures two-way analysis of variance ($p<0.05$). All treatments were effective in reducing dentin Lp compared with the control immediately after the application. However, only potassium oxalate and NaF varnish significantly differed from placebo varnish ($p<0.0001$). The same results were found after the erosive challenge. Therefore, the TiF_4 was less effective than the NaF varnish and potassium oxalate gel in reducing dentin permeability. Using this experimental model, both NaF varnish and potassium oxalate gel reduced the Lp similarly to the presence of smear layer.

INTRODUCTION

Dentin hypersensitivity (DH) afflicts many people.[1] This painful clinical condition often occurs because of dentin exposure resulting from continuous loss of tooth substance as a result of such wear processes as erosion, abrasion, attrition, and abfraction as well as gingival recession. The DH mechanism is still uncertain, although the most accepted hypothesis is the hydrodynamic theory.[2] According to this theory, any decrease in dentinal fluid movement (conductance) should result in reduced DH.[3] Many products have been developed to alleviate this clinical condition, such as potassium oxalate, fluoride, laser, and other agents with dentin occlusive effects.[4,5]

The action of potassium oxalate in diminishing DH is well known. This desensitizing agent obliterates the dentinal tubules by the precipitation of calcium oxalate crystals.[4,6] However, it seems that the crystals formed by the reaction between potassium oxalate and hydroxyapatite are dissolved over time.[7] Additionally, the potassium oxalate does not have any effect against continual dentin erosive wear.

Sodium fluoride (NaF) is one of the most commonly used agents in the treatment of DH.[8,9] Its mechanism of action is attributed to the precipitation of calcium fluoride (CaF_2) on the tooth surface.[4] This layer acts as a mechanical barrier obliterating the opening of the dentinal tubules,[9] minimizing the contact between liquids and dental structure and, consequently, reducing DH.[8,9] Topical fluoride application has also been investigated as a way to decrease the tooth erosive wear,[10] which might be related to the development and progression of DH.[11]

Other fluoride salts besides NaF (eg, titanium tetrafluoride [TiF_4]) have been tested. *In vitro* and *in situ* studies showed that TiF_4 was efficient in minimizing the enamel and dentin erosion compared with other fluoride salts.[12-16] The protective effect of TiF_4 has been attributed not only to the fluoride but also to the titanium that can be immobilized near the surface region of apatite and bonded to the phosphate group, resulting in an acid-resistant metallic layer on the tooth surface.[10,17-19]

Despite the favorable results TiF_4 shows in reducing dentin erosion, its effect in diminishing DH is unknown because only a few studies have been conducted. It has been demonstrated that 0.1% to 1 % TiF_4 solutions have acid-resistant properties to reduce dentin permeability compared with NaF.[20] It also achieved a high level of dentin desensitization (in 75% of patients) when applied *in vivo*.[21]

Therefore, this study compared the effect of TiF_4 varnish and solution, NaF varnish and solution, and potassium oxalate gel on dentin permeability by using hydraulic conductance (Lp) measurements. The tested null hypotheses were that there are no significant differences among the treatment groups and that the control/placebo varnish on the Lp changes 1) after the treatment and 2) after the erosive challenge.

METHODS AND MATERIALS

Experimental Design

This *in vitro* study involved the analysis of two factors: treatment (in seven levels) and dentin condition (in four levels). The response variable was the measurement of Lp.

Tooth Selection and Specimen Preparation

After the Ethics Committee from Bauru School of Dentistry – University of São Paulo, Brazil (Protocol 58/2010) approved the study and the patients signed the informed consent for teeth donation, 220 nonerupted human third molars were obtained. Immediately after the extraction, teeth were stored in 0.02% NaN_3 (sodium azide) solution (Merck, Darmastadt, Germany) at 4°C for a maximum of 6 months. The teeth were sectioned with a diamond disk (XL-12205, Extec Corporation, Enfield, CT, USA) in an Isomet machine (Buehler Ltd, Lake Bluff, IL, USA) at low speed (300 rpm) and under deionized water irrigation. The crown of each tooth was sectioned transversely above the projection of the pulp horns and below the occlusal amelodentinal junction. Only one dentin disk, 1.0 ± 0.2 mm thick, was obtained from the middle third of each molar's crown. The thickness of the disks was controlled using a digital micrometer (Starrett Indústria &

Comércio Ltda, São Paulo, Brazil). A stereoscopic magnifying lens (Meji Techno Co Ltda, Tokyo, Japan) was used to verify whether the disks' surfaces were free of enamel and pulp horn. Thus, 98 dentin disks were obtained in ideal conditions and were stored in deionized water at 10°C until the experiment.

Fluid Flow and Lp Measurements

The rate of fluid flow was obtained using a Flodec machine (DeMarco Engineering, Geneva, Switzerland), as described earlier,[5] for the specimen's selection and distribution proposal and for measuring Lp on the different experimental conditions. A plexiglass split-chamber device was used to connect the dentin disk to the Flodec, allowing the standardization of the dentin surface area (0.282 mm^2) defined by the O-ring through which the fluid passes, under a constant deionized water pressure of 140 cm (2 psi).

The movement of the bubble inside a capillary glass (inside diameter, 0.83 mm; outside diameter, 4 mm; detectable volume by step, 2.71 nL) in the Flodec system was recorded for 5 minutes. The last 3 minutes was used to obtain the Lp for each experimental condition described in the following sections.

Selection of the Specimens

Both sides (occlusal and pulpal) of the 98 dentin disks were first etched by immersion in a 37% phosphoric acid solution for 15 seconds and rinsed in deionized water to ensure maximum permeability. After that, the selected disks were drafted using SPSS program version 13.0 (SPSS Inc, Tulsa, OK, USA). Next, 56 disks were randomly distributed to seven groups (n=8) corresponding to the experimental treatments and controls, so that the mean baseline permeability values were not statistically significant different among the groups (analysis of variance [ANOVA]; $p>0.05$).

Experimental Procedures and the Treatments

Four measures of the Lp were performed for each dentin disk, followed by these conditions: 1) in the presence of the smear layer created by polishing the occlusal side of the dentin disks with 600-grit SiC abrasive paper (Buehler Ltd), which represented minimum permeability; 2) after the removal of the occlusal smear layer with 37% phosphoric acid gel for 15 seconds to obtain maximum permeability; 3) after application of the tested materials (Table 1) (treatment permeability); and 4) after an erosive challenge with 6% citric acid (Merck), pH 2.1, for 1 minute (final permeability).

Details about the treatments are displayed in Table 1. Before application of the materials on the surface of each dentin disk, the disk was gently dried with an absorbent paper. All materials were applied for 4 minutes, and the dentin disk surfaces were washed with deionized water for 30 seconds. Fluoride solutions were applied using a pipette (v=0.2 mL/sample). The varnishes were applied with a microbrush and removed with a probe, taking care not to touch the dentin surface. Potassium oxalate was applied with a cotton swab.

Statistical Analysis

GraphPad InStat version 2.0 for Windows and GraphPad Prism software version 4.0 for Windows (Graph Pad Software, San Diego, CA, USA) were used to perform the statistical analysis. The assumptions of equality of variances and normal distribution of errors were checked for all the variables tested using the Bartlett and Kolmogorov-Smirnov tests, respectively. Because the assumptions were satisfied, repeated measure two-way ANOVA (variables = treatment and dentin condition) followed by Bonferroni post hoc test were used. The significance level was set at $\alpha=0.05$.

RESULTS

Mean values and standard deviations of Lp measurements (percent of Lp reduction compared with the maximum Lp) of the dentin disks treated with the tested materials are displayed in Table 2. Repeated measures two-way ANOVA revealed significant intragroup (criteria = dentin conditions; $p<0.0001$) and intergroups (criteria = the treatments; $p<0.001$) differences and interactions between them ($p<0.001$).

Mean minimum and maximum Lp values within groups differed significantly from each other. Treatment Lp means were similar to minimum Lp values for NaF varnish and potassium oxalate gel only. The same results were found after the erosive challenge: the final Lp means for NaF varnish and potassium oxalate gel were still similar to the minimum values. For the other treatments, the treatment Lp and final Lp values were significantly higher than the minimum Lp values.

All tested materials were effective in reducing the dentin Lp compared with the control immediately after treatment (treatment Lp). After the erosive

Table 1: *Experimental Materials Applied on the Occlusal Surface of Dentin Disks*

Material	Manufacturing	Components	pH
Control	—	—	—
Potassium oxalate gel	Ativus Farmacêutica, Valinhos, SP, Brazil	3% Potassium oxalate monohydrate carboxymethylcelulose	4.1
NaF solution	Sigma-Aldrich, St Louis, MO, USA	5.42 % NaF (2.45% F, pH adjusted with 5 M H_3PO_4 solution)	5.0
TiF_4 solution	Sigma-Aldrich	4% TiF_4 (2.45% F)	1.0
NaF varnish	FGM-Dentscare, Joinvile, SC, Brazil	5.42% NaF (2.45% F, synthetic resin, pH adjusted with 5 M H_3PO_4 solution)	5.0
TiF_4 varnish	FGM-Dentscare	4% TiF_4 (2.45% F, synthetic resin)	1.0
Placebo varnish	FGM-Dentscare	Synthetic resin without fluoride	5.0

challenge (final Lp), all treatments were also found to be effective in maintaining the reduced dentin Lp compared with the control. However, only potassium oxalate and NaF varnish significantly differed from placebo varnish in both conditions. No differences were found among NaF, TiF_4, and potassium oxalate.

DISCUSSION

Almost all desensitizing agents occlude tubules by salt precipitation or resin deposition. Therefore, partial or total tubule obstruction could reduce fluid movement and consequently, reduce the DH.[4] Accordingly, this study applied a method to measure fluid movement *in vitro*.

A method to register dentin fluid movement is the assessment of Lp by using Flodec.[4,5,22] One limitation of the method is that the etched dentin disks, when kept in deionized water for more than 1 week, partially lose their maximum permeability. Because of this, a large number of teeth must be collected (in our case, 220 teeth) to achieve the expected number of samples for a limited time of storage (n=8 per group; 56 disks). Other aspects can also influence the selection of the samples, such as the size and thickness of the dentin disk and the absence of enamel and pulp horn.

Although the maximum Lp represents the opened dentin tubules, the minimum Lp is the permeability of the smear layer (eg, nearly no permeability). The desensitizing agent is considered effective when the Lp values achieved are similar to those found in the presence of smear layer.

This study mainly focused on TiF_4 treatment, as it is considered a potential anticariogenic and antierosive agent.[10] Because of its mechanism of action,[17-19] it was thought that this metallic fluoride could occlude dentin tubules and reduce DH.[20,21] However, only potassium oxalate and NaF varnish were able to significantly reduce the dentin Lp compared with the control and placebo varnish. Both treatments were potentially effective as occlusive agents, as they achieved Lp values (treatment and final Lp means) similar to the minimum values. Therefore, the null hypotheses of this study were rejected.

Table 2: *Mean (Standard Deviation) of the Lp Values ($\mu l\ c^{-2}\ min^{-1}\ cm\ H_2O$) of the Different Treatment Groups Under Four Experimental Dentin Conditions (n=8)[a]*

	Minimum (With Smear Layer)*	Maximum (After Acid etching)	Treatment*	Final (After Erosive Challenge)*
Control	1.4 (1.8)^{Aa} (93.9%)	22.8 (14.6)^{Ba}	22.0 (9.7)^{Ba} (3.5%)	24.2 (7.2)^{Ba} (−6.0%)
Potassium oxalate	1.4 (1.8)^{Aa} (94.5%)	25.5 (12.2)^{Ba}	3.9 (3.0)^{Ac} (84.7%)	5.6 (4.0)^{Ac} (79.1%)
NaF solution	0.6 (0.6)^{Aa} (96.9%)	19.7 (8.7)^{Ca}	10.0 (6.1)^{Bc} (49.2%)	13.6 (6.1)^{BCbc} (31.0%)
TiF_4 solution	1.8 (0.9)^{Aa} (89.7%)	17.5 (7.9)^{Ba}	11.3 (10.1)^{Bbc} (35.4%)	11.3 (7.4)^{Bbc} (35.4%)
NaF varnish	1.4 (0.8)^{Aa} (93.0%)	19.9 (8.6)^{Ba}	6.9 (3.5)^{Ac} (65.3%)	7.6 (3.8)^{Ac} (61.8%)
TiF_4 varnish	0.7 (0.5)^{Aa} (96.0%)	17.6 (8.1)^{Ba}	11.0 (4.0)^{Bbc} (37.5%)	3.6 (5.8)^{Bbc} (22.7%)
Placebo varnish	1.1 (0.9)^{Aa} (94.5%)	20.1 (4.4)^{Ba}	18.7 (7.8)^{Bab} (7.0%)	17.6 (7.8)^{Bab} (12.4%)

[a] *Mean percent of Lp reduction compared with the maximum Lp values. In the same row (intragroup comparison), different uppercase superscript letters indicate significant difference among the experimental conditions for each treatment separately ($p<0.05$). In the same column (intergroups comparison), different lowercase superscript letters indicate significant differences among the treatments for each condition individually ($p<0.05$) (repeated measures two-way ANOVA: the experimental conditions, $p<0.0001$; the treatments, $p<0.001$; and the interaction between them $p<0.001$).*

The positive effect of potassium oxalate is attributed to the formation of a insoluble salt of calcium oxalate that can precipitate and penetrate the dentin tubules, partial or totally obliterating them and reducing the dentin permeability[23] and consequently the DH.[4,24] The gel is able to penetrate the tubule and react with calcium ions from the dentin fluid to form insoluble calcium oxalate crystals.[4]

On the other hand, the mechanism of action of the conventional fluoride (NaF) occurs by the precipitation of CaF_2 on the dental surface, which apparently acts as a mechanical barrier,[9] which could also affect dentin permeability and DH.[8] However, clinical studies have shown that its antihyperesthesia effect is of short duration.[25] Among the NaF agents, only the varnish was able to maintain an Lp similar to the minimum Lp after an erosive acid attack. It might be speculated that NaF varnish created more stable crystallization retention inside the dentinal tubules than the NaF solution.

Corroborating the findings, previous studies have shown that TiF_4 solution created a precipitated layer on peri- and intertubular dentin with partial or no tubule covering. Nothing has been seen inside the tubules.[10,15] The present study was developed on dentin without a smear layer, and TiF_4 was unable to reduce Lp as the smear layer. Kazemi and others[20] showed that TiF_4 applied on dentin containing smear layer reduced dentin permeability after an erosive attack. Therefore, it might be speculated that the formation of the TiF_4 glaze layer on a dentin surface is modulated by the presence of a smear layer. Because of the low pH of the TiF_4, its interaction with the smear layer might allow the precipitation of the layer throughout the dentin surface, which would prevent not only erosion, as shown by previous studies,[10] but probably the DH as well.[21] Futures studies should be done to test the effect of TiF_4 on Lp values when applied on dentin in the presence of a smear layer, as happens in a clinical situation.[21]

Despite the ineffectiveness of TiF_4, the samples treated with this fluoride salt did not show an increase in Lp values after erosive challenge. Preservation of Lp may be attributed to some superficial precipitation, which does not behave like the smear layer at all.

One important point to be considered in interpreting the data is that the varnish usually remains for a longer time on the tooth surface than the solution. Therefore, the results of this *in vitro* study might not be directly extrapolated to clinical conditions, where the contact of the varnish with the tooth lasts longer, and the saliva might have interplay on the DH process and the reaction between the fluoride salt and the tooth. Accordingly, future studies should be done to test the performance of the experimental agents against DH in a clinical trial.

The longevity of the precipitates or resins on or inside dentinal tubules and their ability to resist acid challenge over time are also unknown. It has been shown that oxalate precipitates appear to wash out under challenge in the clinical environment, and the effect of NaF is reduced after 3 and 6 months of application.[25] Considering that the clinical effectiveness of these materials depends in part on the dissolution resistance or solubility level of precipitates or resins, new studies should be done, including repetitive erosive challenges.

CONCLUSIONS

In conclusion, TiF_4 was less effective than NaF varnish and potassium oxalate gel in reducing dentin permeability using this experimental model. The NaF varnish and potassium oxalate gel seem to be good materials to reduce dentin permeability and, consequently, DH.

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Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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