A warmed topical fluoride solution enhances KOH-soluble and -insoluble fluoride formation on tooth surfaces *in vitro*

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Key Words

KOH-soluble fluoride; KOH-insoluble fluoride; Topical fluorides; Temperature; Acidified Phosphate Fluoride (APF).

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Abstract

In paediatric dentistry, topical application of fluoride is a vital routine preventive procedure. Therefore, it is important to identify more effective methods of fluoride application. This study investigated the effect of warmed topical fluoride solutions on human enamel. Several solutions (acidified phosphate fluoride (APF), 2% NaF or 0.2% NaF) were applied to human premolars for 5 min at 25, 37, 50, and 60°C. KOH-soluble and -insoluble fluoride formation on the enamel surface was then measured. KOH-soluble fluoride formation increased proportionally with temperature. KOH-soluble fluoride levels at 60°C were 1.7-, 2.6-, and 2.7-fold greater than at 25°C for 0.2% NaF, 2% NaF, and APF, respectively. Also, KOH-insoluble fluoride incorporation into the enamel increased proportionally with the solution temperature. KOH-insoluble fluoride levels at 60°C were 4.9-, 7.5-, and 2.8-fold greater than at 25°C for 0.2% NaF, 2% NaF, and APF, respectively. The dissolution of enamel in APF at the time of topical application is a major clinical concern. Therefore, the dissolved calcium in the APF solution was assayed and found to be equivalent to enamel thicknesses of 0.007, 0.042, 0.045, and 0.05 μm respectively. The effect of increasing the temperature outside the tooth on the pulp temperature was monitored for 5 min. Pulp temperature increased by 3 and 8°C using solutions at 50 and 60°C, respectively, which was considered to be unlikely to have adverse effects on the pulp. The warming of topical fluoride solution, even slightly, can be a useful method to make the topical fluoride application more effective.

1. Introduction

Fluoride is currently recognised as the main factor responsible for the significant decline in the prevalence of dental caries that has been observed worldwide [1]. Periodic topical fluoride treatments are recommended for children and adults who are at moderate or high risk of developing caries according to the guidelines set forth by the American Dental Association [2]. Two meta-analyses of clinical studies have shown the overall caries-inhibiting effect of APF gel to be 21% (95%, CI = 14-28%) [3] and 22% (95% CI = 18-25%) [4]. Jiang et al. [5] reported that the number of decayed, missing, or filled tooth surfaces (dmfs) was significantly reduced, by 24%, in primary teeth after bi-annual professional application of APF foam during a 2-year period.

Two mechanisms have been proposed to explain the effectiveness of topical fluoride [6]. The first is incorporation of KOH-insoluble fluoride compounds into the enamel. Alternatively, calcium-fluoride-like precipitates, composed of KOH-soluble fluoride, may form deposits on the enamel surface. Indeed, fluoride-enriched enamel showed reduced demineralisation proportional to the enamel fluoride concentration in a thin enamel section [7]. Enamel crystallite dissolution is maximally inhibited when completely covered by adsorbed fluoride derived from KOH-soluble fluoride [8]. Greater protection against acid can be achieved when both KOH-soluble and -insoluble fluoride are applied to enamel.

A simple method of accelerating fluoride incorporation into enamel is increasing the temperature of the solution. Sheinin [9] reported that compared with treatment at 15°C, the concentration of fluorine increased by 3.6 and 6.3 fold after treating the enamel powder with a 2% NaF solution for 10 min at 37 and 60°C, respectively. According to Mellberg and Loertscher [10], changing the treatment temperature from room temperature to 35°C approximately doubled the uptake of fluoride by intact surface human enamel from a topical APF solution. Putt et al. [11] reported significant increases in fluoride and tin uptake by enamel via treatment with SnF_2 at 25, 45, 65 and 85°C. However, all these papers investigated only the fluoride incorporation into enamel and did not pay attention to the calcium fluoride-like precipitation that can be measured as the KOH-soluble fluoride.

The temperature of the fluoride gel or foam used in daily topical fluoride application in dental practice is usually not a matter of concern to dentists. However, it would be advantageous if the effectiveness of topical fluoride application could be improved by increasing the temperature. The aim of this study was to investigate the effect of increasing the temperature of topical fluoride on the formation of both KOH-soluble and -insoluble fluoride on the enamel surface.

2. Materials and Methods

2.1. Human teeth

One hundred and twenty human premolars were used in this study. The teeth were extracted for orthodontic reasons and stored in an atmosphere of 100% humidity at 4°C until use. The tooth surface was examined under a microscope and only teeth without cracks, caries, or white spots were selected. The tooth root was removed, and the tooth was then embedded in self-polymerising resin (Unifast A3, GC Company Tokyo Japan) with the buccal enamel surface exposed. The surface was polished using sand paper (CH2-HX＃1000, Noritake Coated Abrasive), and the mesial or distal half of the polished surface was used as a test or a control. The exposed fresh enamel surface was rinsed in ultra-pure water using an ultrasonic cleaning device. Ethics approval was obtained from the Ethics Committee of Iwate Medical University, Japan (No. 01180).

2.2. Fluoride treatment

Each specimen was rinsed with ultra-pure water and blotted dry before fluoride treatment. The teeth were individually treated for 5 min with agitation in separate plastic beakers, each containing 10 ml of solution. Three fluoride solutions: 0.2%NaF (900 ppmF・pH 7.0), 2% NaF (9000 ppmF・pH 7.0), APF (9000 ppmF・pH 3.5), were pre-heated to the appropriate temperature (25, 37, 50, and 60°C) within ± 1°C in a thermostatically controlled chamber. The pH of each solution was measured using a flat ISFET pH electrode (0040-10D, Horiba, Kyoto, Japan).

2.3. Assay of KOH-soluble fluoride

After rinsing for 1 min in a stream of ultra-pure water, the tooth sample was immersed with agitation at room temperature for 24 h in 10 ml (for the 2% NaF and 0.2% NaF samples) or 40 ml (for the APF sample) of 1 M KOH to remove loosely bound fluoride from the tooth surface, following the methodology of Caslavska et al. [12]. The fluoride ion concentration was measured using a fluoride-ion-selective electrode (9609BNWP, Thermo Scientific, Waltham Ma USA) in a 0.5-ml aliquot, to which 0.5 ml TISABII buffer (Thermo Scientific, Waltham Ma USA) was added. KOH-soluble fluoride was quantified as fluoride (μg) per unit exposed enamel surface area (cm^2), following the methodology of Dijkman et al. [13].

2.4. Assay of KOH-insoluble fluoride

After the loosely bound fluoride had been removed and measured, the fluoride concentration of the surface enamel was analysed by enamel biopsy following a modified method of Kadoma et al. [14]. A piece of adhesive tape with a 2-mm-diameter window was placed on the surface of the enamel specimen. Discs 4 mm in diameter and 1 mm thick were punched out from glass-fibre filter paper (GA100, Toyo Roshi Co. Ltd., Tokyo, Japan). Four successive enamel layers were etched from the window area by pipetting 5 μl of 6 M HCl onto the discs. After 30 s of etching, the HCl solution remaining on the enamel window was blotted twice, and all three discs were placed in 1 ml of ultra-pure water. The water was then subjected to assays for fluoride and calcium. The fluoride concentration was measured using the fluoride-ion-selective electrode as for KOH-soluble fluoride. The calcium concentration was measured using a calcium-ion-selective electrode (6583-10C, Horiba, Kyoto, Japan) in 0.5 ml of sample added to 0.5 ml of ultra-pure water, 128 μl of 0.1 M NaOH and 20 μl of ionic-strength-adjusting buffer (ISA, Thermo Scientific, Waltham Ma USA). The final aliquot volume was 1148 μl, with a pH of 7.0.

The weight and volume of enamel removed by each acid etch and the corresponding fluoride concentration were calculated using values of 2.95 for human enamel density and 37% for calcium content. We estimated the thickness of the enamel layer by dividing the enamel volume by the area of the exposed window (3.14 mm^2). The fluoride concentration profile for the surface enamel was drawn using four continuous data points. The fluoride concentration at 10 μm from the enamel surface was calculated by the interpolation of two data points at depths greater and less than 10 μm.

2.5. Assay of calcium dissolution by the APF solution

To quantify the calcium dissolved from the enamel surface by APF application, the calcium concentration in each fluoride solution post-application was measured using a calcium-ion-selective electrode in 0.5 ml of sample, to which 0.5 ml of 0.1 M NaOH and 20 μl of ISA were added.

2.6. Heat transmission in human teeth

To investigate the effect of exposure to a heated solution, changes in pulp temperature were monitored for 5 min *in vitro*. Five premolars with the root removed were used. A thermocouple probe (No. 900-23, Yokogawa Company, Tokyo, Japan) was inserted into the pulp chamber, and covered and fixed with self-curing resin. The crown of the tooth was dipped into a 25°C water bath until the pulp temperature reached 25°C. The specimen was then immersed in a water bath at 50 or 60°C, and the temperature of the pulp in the crown area was monitored for 5 min.

2.7. Statistical analysis

SPSS for Windows (Apache Software Foundation, 2001, USA) was used to statistically analyse the data. Student's t-test was used to evaluate significance, which was set at 5% for all tests.

3. Results

3.1. KOH-soluble fluoride

KOH-soluble fluoride levels on the enamel after a 5-min application of 0.2% NaF, 2% NaF, and APF at 25, 37, 50, and 60°C are shown in Fig. 1. The formation of KOH-soluble fluoride on the enamel surface increased proportionally with the increase in solution temperature. KOH-soluble fluoride levels at 60°C were 1.7-, 2.6-, and 2.7- fold greater than at 25°C for 0.2% NaF, 2% NaF, and APF, respectively. The ratios of KOH-soluble fluoride formed by 2% and 0.2% NaF were 1.6, 2.1, 2.2, and 2.4 at 25, 37, 50, and 60°C, respectively. Also, KOH-soluble fluoride levels after application of APF were 6.9-, 9.0-, 6.9, and 7.2-fold greater than those after application of 2% NaF at 25, 37, 50, and 60°C, respectively.

3.2. KOH-insoluble fluoride

The fluoride levels 10 μm from the enamel surface after a 5-min application of 0.2% NaF, 2% NaF, and APF at 25, 37, 50, and 60°C are shown in Fig. 2. The incorporated fluoride increased proportionally with solution temperature. KOH-insoluble fluoride levels at 60°C were 4.9-, 7.5-, and 2.8-fold greater than at 25°C for 0.2% NaF, 2% NaF, and APF, respectively.

3.3. Calcium dissolution from the enamel surface by APF application

Table 1 shows the amounts of calcium dissolved from the enamel surface at 5 min post-APF application. The APF solution dissolved significantly more calcium at 37°C than at 25°C; however, little calcium was dissolved at either temperature. Increasing the temperature from 37°C to 50 or 60°C did not significantly affect calcium dissolution from the enamel.

3.4. Temperature change in the pulp

Fig. 3 shows the changes in pulp temperature over time after the tooth was dipped in water at 50 or 60°C. This may mimic the effect on pulp temperature of immersion in a fluoride solution at 50 or 60°C. The pulp temperature increased to almost 40°C after 100 s and 45°C after 50 s when the tooth was immersed in a water bath at 50 or 60°C.

4. Discussion

Modest amounts of KOH-soluble fluoride were formed on the enamel by application of 2% and 0.2% NaF, and the amount increased proportionally with solution temperature (Fig. 1). KOH-soluble fluoride, considered to be a CaF_2-like material, is deposited on the enamel surface by reacting with the Ca dissolved from the enamel [15]. The 10-fold difference in fluoride concentration between 2% NaF and 0.2% NaF resulted in an approximately twofold increase in KOH-soluble fluoride formation. This likely reflects the quantity of calcium released from the enamel surface. In contrast, considerably more KOH-soluble fluoride was formed by APF application than by the neutral 2% NaF solution, despite the identical fluoride concentration. Also, KOH-soluble fluoride formation by APF was enhanced as the temperature increased (Fig. 1). The two solutions differ in the presence of phosphoric acid, which leads to increased production of KOH-soluble fluoride.

Dissolution of enamel by APF at the time of topical application as a preventative procedure is of concern to clinicians. Brudevold et al. [16], who developed an APF solution for caries prevention, reported that limited calcium dissolution occurred. In this study, the levels of calcium dissolution by the APF solution were 0.008, 0.045, 0.049, and 0.054 $\mu g/cm^2$ at 25, 37, 50, and 60°C, respectively. This is equivalent to 0.007, 0.042, 0.045, and 0.05 μm of enamel. These values are within the permissible range compared to that caused by the phosphoric-acid-etching procedure at the time of composite resin or resin sealant application, which is in the order of 5 to 10 μm [17].

The average increase in fluoride incorporation into enamel was enhanced as the temperature increased. Fluoride incorporation for APF was 2.8-fold greater at 60°C than at 25°C, and 7.5-fold greater with 2% NaF. No significant difference was found between 50 and 60°C for APF. This may be because the fluoridated hydroxyl apatite formed at the enamel surface at 50°C prevents further dissolution of enamel apatite, so further precipitation is inhibited. The difference in fluoride incorporation between 2% NaF and APF is due to the effect of phosphoric acid in APF. This leads to dissolution of enamel apatite and supply of Ca^{2+} and PO_4^{3-}, which re-precipitates on the enamel surface as fluoridated hydroxy apatite [16].

Currently, structurally incorporated fluoride is considered less effective in terms of inhibiting demineralisation [8], based on the work of Øgaard et al. [18], who placed human and shark enamel slabs in removable appliances and covered them with orthodontic bands to allow plaque accumulation. Microradiographic analyses showed that carious lesions formed even in shark enamel, which is composed of pure fluoroapatite. However, the depth of the lesions was 60% less than those in the human slab, and mineral loss was 43% less. Daily rinsing with 0.2% NaF was shown to be as protective as or more protective than the shark enamel.

These results suggest that structurally bound fluoride does not strongly inhibit demineralisation following caries attachment to the tooth surface. Conversely, Tanaka et al. [7] reported that fluoride enrichment of human enamel resulted in reduced demineralisation, which was inversely related to the enamel fluoride content. Takagi et al. [19] also showed that enamel resistance to lesion formation increased with tooth-bound F content. Thus, a higher enamel fluoride concentration should still be considered advantageous for caries prevention.

Gel, foam, and varnish are used for topical application of fluoride. In addition to the tray method, an alternative drug delivery system has been developed [20]. This system is a custom-made tray prepared using thermoplastic resin, and can be used for application of not only fluoride solution but also a bacteriostatic solution to reduce the number of mutans streptococci. Our preliminary study indicated a possible use of a modified drug delivery system equipped with silicon tubes for circulation of the solution. This system can supply a fresh source of fluoride to even the inter-proximal area at an arbitrary temperature.

Possible side effects of increasing the solution temperature must be taken into consideration. Transmission of heat through the enamel and dentin was measured using a micro thermometer inserted into the pulp chamber. The pulp temperature increased to 40 and 45°C when the tooth was dipped in a water bath at 50 and 60°C, respectively. Zach and Cohen [21] investigated the histological reaction in dental pulp to an increase in surface temperature using Rhesus monkeys. A soldering iron heated to 275°C was placed on the tooth surface, and the pulp temperature was measured. Histological observation showed that 15% and 60% of teeth, whose temperature increased by 5.5°C and 11.1°C, respectively, suffered irreversible pulp damage. In contrast, Baldissara et al. [22] demonstrated that an intra-pulpal temperature increase of 8.9–14.7°C in human teeth did not produce any pathological change in the pulp. The temperature increases in the pulp in this study were 3 and 8°C for solutions at 50 and 60°C, respectively. If a threshold value of 5.5°C is applied to the data of this study, increasing the temperature of the fluoride solution to 60°C may cause pulpal damage, but increasing the temperature to 50°C is unlikely to exert any adverse effects on the pulp.

5. Conclusion

The production of both KOH-soluble and -insoluble fluoride on enamel surface area was enhanced in proportion to the temperature of the topical fluoride solution. Application of topical fluoride may be made safer and more effective by using a solution at 50°C delivered in a custom-made tray. The warming of topical fluoride solution, even slightly, can be a useful method to make the topical fluoride application more effective.

Disclosure

The authors have no relevant financial relationships or any conflicts of interest to declare.

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Legends for table and figures

Table 1.

Amounts of calcium dissolved from the enamel surface and the estimated enamel layer thicknesses removed by APF application at 25, 37, 50, and 60°C. Each value represents the mean and standard deviation of five tooth samples. The symbol * indicates a significant difference.

Fig. 1.

KOH-soluble fluoride formation on enamel after a 5-min application of 0.2% NaF, 2% NaF, and APF at 25, 37, 50, and 60°C. Each column represents the mean ± standard deviation of 10 tooth samples. A significant difference was observed between columns with different letters ($p<0.05$).

Fig. 2.

Fluoride concentrations 10 μm from the enamel surface after a 5-min application of 0.2% NaF, 2% NaF, and APF at 25, 37, 50, and 60°C, respectively. Each column represents the concentration minus that of the control, expressed as the mean and standard deviation of 10 tooth samples. A significant difference was observed between columns with different letters ($p<0.05$).

Fig. 3.

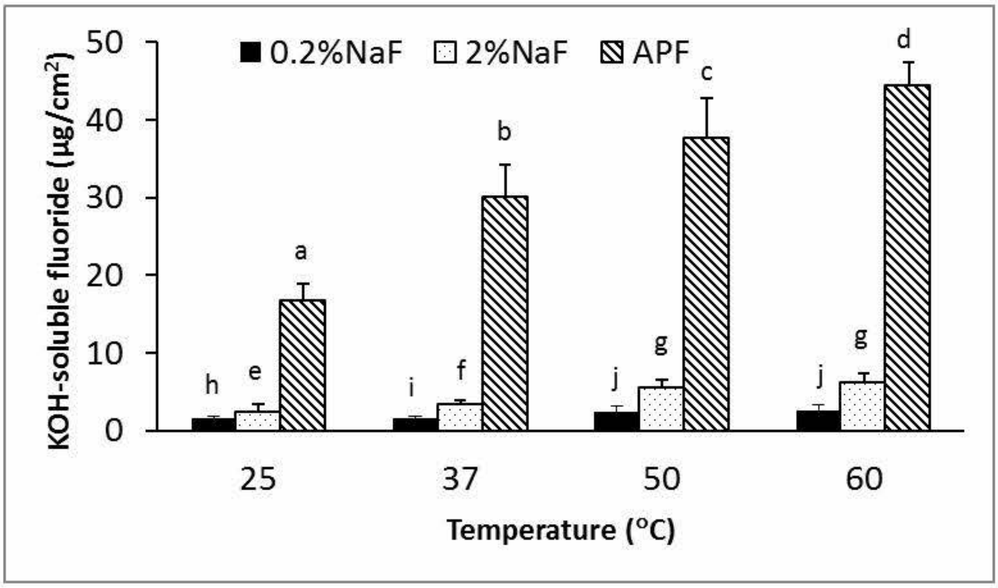
Mean changes in pulp temperature over time after the tooth was dipped in water at 50 or 60°C. Five teeth per temperature were used.

Table 1

	Dissolved calcium (μg/cm²)		Estimated enamel layer removed (μm)	
	Mean (n=5)	SD	Mean (n=5)	SD
25°C	0.008	0.007	0.007	0.006
37°C	0.045	0.025	0.042	0.023
50°C	0.049	0.010	0.045	0.009
60°C	0.054	0.014	0.050	0.013

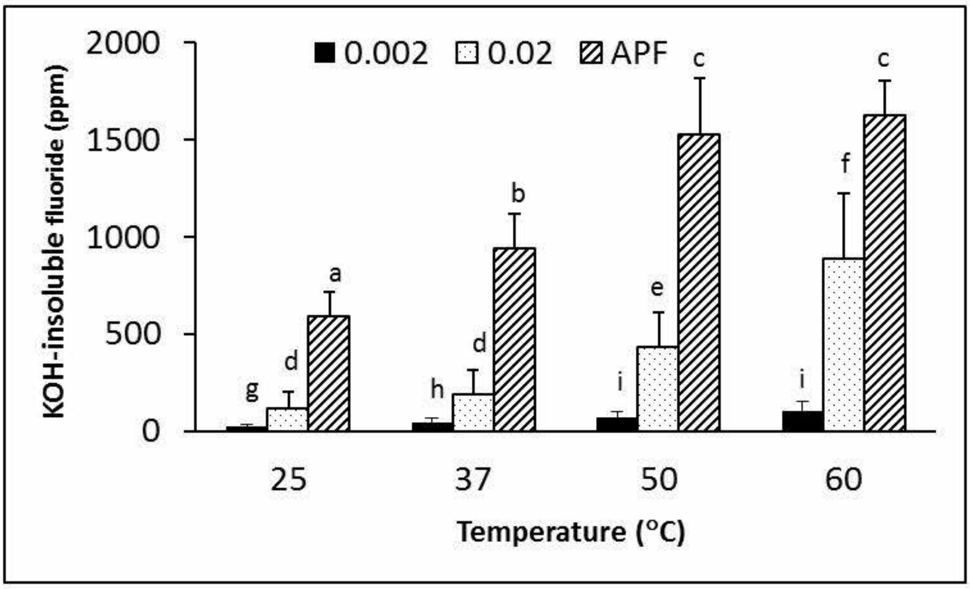
*

Fig. 1



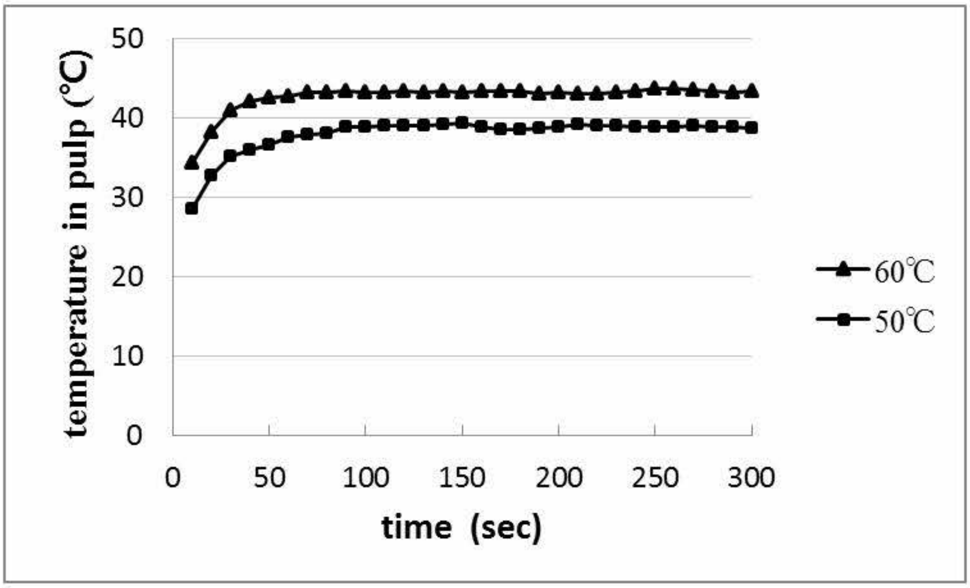
0.2%NaF
2%NaF
APF
KOH-soluble fluoride (µg/cm2)
50
40
30
20
10
0
h
e
a
i
f
b
j
g
c
j
g
d
25
37
50
60
Temperature (°C)

Fig. 2



2000
1500
1000
500
0
0.002
0.02
APF
g
d
a
h
d
b
i
e
c
i
f
c
25
37
50
60
KOH-insoluble fluoride (ppm)
Temperature (°C)

Fig. 3



temperature in pulp (℃)
50
40
30
20
10
0
0
50
100
150
200
250
300
time (sec)
60℃
50℃