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Research Article

Changes in Salivary Free Fluoride Ion Concentration after Adding Low-Concentration Fluoride

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Submitted: 10 May 2016

Accepted: 07 June 2016

Published: 09 June 2016

ISSN: 2333-7133

Keywords

- Salivary free fluoride ion concentration
- Salivary protein
- Calcium
- Caries prevention
- Artificial saliva

Abstract

Fluoride (F) is well known to be effective for caries prevention; however, the safest and most effective method of administration is still unclear. Education regarding efficacy and administration of F is necessary for patients, especially for children and their guardians. In this study, we attempted to elucidate the factors that decrease added F in artificial saliva, and the factors that maintain appropriate salivary F ion concentration for cavity protection. Our original rigorous pilot study determined that the baseline salivary F ion concentration in infants was lower than the free F ion concentration required for caries prevention. The present study aimed to determine the major factors that reduced added F in solution. Organic salivary components (the proteins mucin and albumin) and inorganic salivary components (calcium) were applied in this study and the decrease in F ratios was measured after adding low-concentration F. Consequently, the free F ion concentration in F-added protein and calcium solutions showed significantly lower values compared with that in ultrapure water with the same amount of added F. Furthermore, the free F ion concentration in protein plus calcium solution exhibited a greater decrease after adding low-concentration F. This indicated that there is a synergistic action between protein and calcium when binding with added F in solution.

ABBREVIATION

F: Fluoride

INTRODUCTION

Free low-concentration F ion in saliva has been reported to provide important preventive effects for dental caries. In particular, an enhancement of remineralization and inhibition of demineralization have been reported at salivary free F ion concentrations of 0.014 – 0.02 ppm [1-3]. However, the factors affecting the presence of free F ion in saliva have still not been elucidated.

To evaluate the factors affecting the presence of free F ion in saliva, we obtained and reported data on salivary free F ion levels by measuring the free F ion concentration in the saliva of infants using a flow injection analysis device that can measure very low F ion concentrations [4]. The results of this research revealed the mean salivary free F ion concentration in infants to be 0.0082 ± 0.0026 ppm [5], which is considerably lower than the concentration needed for caries prevention. The results of these studies indicated that added F is immediately bound by salivary substances, resulting in major reduction in salivary free F. Therefore, we concluded that further studies are necessary to confirm the mechanism of reduction. Protein and calcium were selected as major saliva components for the first stage, and their association with F in solution was evaluated. Further, we formulated an artificial saliva solution with protein and calcium to replicate naturally occurring saliva, which contains high amounts of protein and calcium.

This study aimed to investigate the changes in salivary free F ion concentration after adding low concentrations of F and determine the influence of salivary proteins and calcium in solution.

MATERIALS AND METHODS

Specimens

All reagents used in the study were manufactured by Wako Pure Chemical Industries Ltd, and ultrapure water (Milli Q, Millipore, Tokyo, Japan) was used to prepare reagents and buffer solution [4].

1) Calcium solution

Calcium solutions of 0.25, 0.75, and 1.25 mg/100 mL were prepared by dissolving calcium carbonate using diluted hydrochloric acid.

2) Protein solution

Protein solutions (0.3%) were prepared by dissolving mucin from porcine stomach and albumin from bovine serum into phosphate buffered saline (pH 7.2) and dialyzed.

3) Artificial saliva (protein solution with calcium)

To create artificial saliva, 1.25 mg/100 mL of calcium solution was added into each protein solution.

4) F standard solution

Solutions with F ion concentrations of 0.005, 0.01, 0.05, 0.1, and 0.5 mg/L (ppm) were prepared by dissolving NaF powder into ultrapure water. Subsequently, these five solutions were used for standardization.

Measurement of F ion concentration

The flow injection analysis device (FAU2200; Yamato Denshi, Kyoto, Japan) was connected with an F electrode cell (Model 94-09, Orion) and used to measure the F ion concentration. The peak values of electric potential differences were measured using the device, and a standard curve was prepared. Free F ion concentrations in solution were measured according to the peak height. All specimens were measured 5 min after the addition of low-concentration F.

1) F standard solutions of 0.05, 0.1, or 0.5 ppm were added into 0.3% mucin or albumin solution and ultrapure water, respectively, at a ratio of 1:9 (F standard solution: other solution).

2) F standard solution of 0.5 ppm was added into 0.25, 0.75, or 1.25 mg/100 mL of calcium solution and ultrapure water at a ratio of 1:9 (F standard solution: other solution).

3) After adding F standard solution of 0.5 ppm to mucin or albumin artificial saliva and ultrapure water at a ratio of 1:9 (F: other solution), changes in free F ion concentration in each specimen were measured.

Statistical processing

The F ion concentrations in calcium solutions were compared using one-way analysis of variance and parametric multiple comparisons with Tukey's honestly significant difference test. Furthermore, t-tests were used in each combined protein and calcium solution with the significance level set at 5% ($p < 0.05$). The decrease ratio was represented in percent by the decreased amount with respect to the F ion concentration (0.05 ppm) in ultrapure water. All analyses were conducted with statistic calculation software SPSS 21.0 J for Windows (IBM, Tokyo, Japan).

RESULTS AND DISCUSSION

Results

1) Free F ion concentration in calcium solution after adding F (Table 1 and Figure 1).

(1) The decreased ratios in 0.25, 0.75, or 1.25 mg/100 mL of calcium solution with added F were lower compared with that of ultrapure water with the same amount of added F.

(2) The free F ion concentration in calcium solutions with added 0.5 ppm of F was reduced in proportion to the amount of calcium.

(3) The decrease in the ratio of free F ion concentration in each calcium solution was 9.6%–34.6%, and free F ion concentration in 1.25 mg/100 mL of calcium solution showed the maximum percentage decrease. There was a significant difference between 0.25 and 1.25 mg of calcium content.

2) Free F ion concentration in protein solution after adding F (Table 2).

(1) Mucin solution with added F showed a significantly low level of free F ion concentration compared with that in ultrapure water with the same amount of added F.

Table 1: Measurements of free F ion concentrations after adding 0.5 ppm of F to 0.25, 0.75, or 1.25 mg/100 mL of calcium solutionand to protein solutions with 1.25 mg/100 mL of calcium solution.

Unit: ppm		
Added F ion level		0.5
Ultrapure water		0.05[a, b, c, d, e]
0.25mg/100 mL of calcium solution	Mean	0.04520[a, *]
	SD	0.00262
	Decrease	9.6%
0.75 mg/100 mL of calcium solution	Mean	0.03935[b]
	SD	0.00492
	Decrease	21.3%
1.25 mg/100 mL of calcium solution	Mean	0.03270[c, *]
	SD	0.00170
	Decrease	34.6%
Mucin solution + 1.25 mg/100 mL of calcium solution	Mean	0.01893[d, **]
	SD	0.00211
	Decrease	64.14%
Albumin solution + 1.25 mg/100 mL of calcium solution	Mean	0.02760[e, **]
	SD	0.00462
	Decrease	44.8%

$p < 0.05$
Decreased ratio is represented in percent by comparing the decreased amount with the F ion concentration in ultrapure water.
[a, b, c, d, e, *, **]Statistically significant differences between groups.
Abbreviations: F: Fluoride; SD: Standard Deviation

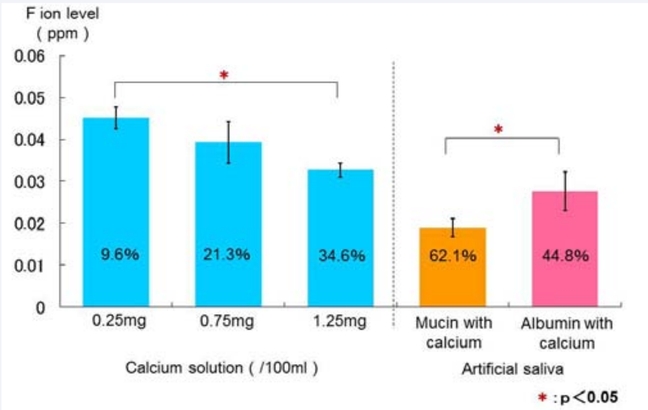

Figure 1 Measurements of free F ion concentration after adding 0.5 ppm of F solution to calcium solution and artificial saliva. The measurements were performed 5 min after adding F solution. Changes in percentage indicate the decreased ratio.

Table 2: Measurements of free F ion concentrations after adding 0.5 ppm of F to 0.3% of protein solution.

Unit: ppm				
Added F ion level		0.05	0.1	0.5
Ultrapure water		0.005[a, b]	0.01[c, d]	0.05[e]
Mucin solution	Mean	0.0040[a]	0.0079[c]	0.0486[e]
	SD	0.0001	9.8E-5	0.0005
	Decrease	18.6%	20.9%	2.8%
Albumin solution	Mean	0.0042[b]	0.0081[d]	0.0495
	SD	0.0002	7.7E-5	0.0007
	Decrease	15.7%	19.1%	1.1%

$p < 0.05$
Decreased ratio is represented in percent by comparing the decreased amount with the F ion concentration in ultrapure water.
[a, b, c, d, e] Statistically significant differences between groups.
Abbreviations: F: Fluoride; SD: Standard Deviation

(2) Albumin solutions with 0.05 and 0.1 ppm of added F showed a significantly low free F ion concentration.

(3) There was no significant difference in the free F ion concentrations between mucin and albumin solutions with added F.

(4) The decrease in the ratio of F ion concentration in protein solution compared with that in ultrapure water was 1.1%–20.9%.

3) Free F ion concentration in mucin or albumin artificial saliva (Table 1 and Figure 1).

(1) The decrease in the ratio of free F ion concentration in mucin artificial saliva compared with that in ultrapure water was 62.1%.

(2) The decrease in the ratio of free F ion concentration in albumin artificial saliva compared with that in ultrapure water was 44.8%.

(3) There was a greater reduction in free F ion concentration in mucin artificial saliva than in albumin artificial saliva.

Discussion

There are several organic and inorganic components in saliva, and it is difficult to clarify the details due to its diverse composition. Therefore, there is a wide range of data regarding the types and amount of protein contained in saliva [6]. In this study, mucin and albumin were chosen because they are the typical salivary proteins and evaluated to determine their binding characteristics with low-concentration F.

Mucin is secreted by submandibular glands and influences the degree of salivary viscosity. Even though albumin concentration is very low in saliva than that in blood plasma, it is related to the stabilization of salivary protein form. To determine the concentration of salivary protein, we applied the mucin concentrations according to the existing reports of the studies by Fox et al [7] and Tajime et al [8]; however, it has been reported that protein concentrations in saliva differ with respect to subject selection, individual variability, measurement methods, etc. As previously mentioned, though saliva contains albumin, it is impossible to recreate salivary albumin concentrations because they are present in extremely small amounts (1%) in the saliva [9]. The purpose of this study was to confirm the binding characteristics of salivary proteins with added F. Hence, the concentrations of mucin and albumin were set for descriptive purposes along with close to actual salivary mucin concentrations [7,8].

This study results indicate that a proportion of salivary free F ions have the ability to combine with mucin and albumin. There are several reports that investigated the relationship between salivary components and added F using artificial saliva. However, most of them excluded protein in the artificial saliva; therefore, the outcomes using artificial saliva with proteins has been elusive. In general, studies using protein-added artificial saliva or human saliva are complicated. The inclusion of protein into artificial saliva is useful for research dealing with F in solution because protein, as well as calcium, may possibly reduce the availability of added F substantially. In our study, the decrease in the ratio of free F ion concentrations in F-added protein solution was lower compared with that of 19%– 45% in F-added saliva of infants [5] (Table 3). Furthermore, a larger decrease in the ratio of free F ion concentrations was observed after adding same amount of calcium to protein solution. F binds to the calcium on the tooth surface and creates the substances similar to calcium fluoride crystals depending on the pH level and F concentration [10,11]. F has high electro negativity; therefore, it is indicated that added F bound to calcium not only on tooth surface but also to artificial saliva. However, we must consider the possibility of variations in measurement values because of the very low concentration of added F. Therefore, more number of specimens is required to reveal the relationship between low-concentration F and various saliva components.

To determine the salivary free F ion concentration required to maintain a favorable oral environment [12,13], it is important to clarify the changes in F ion concentrations after in the mouth. From these results, it is believed that elucidating the relationship between F and various saliva components and confirming the changes in salivary free F ion concentration will lead to a safer and more effective use of F for infants in the future.

CONCLUSION

The free F ion concentrations in F-added protein solution and calcium solution showed lower values compared with those in

Table 3: Measurements of free F ion concentrationsafter adding F standard solution to infants' saliva and decrease ratio.

	Unit: ppm		
Added F ion level	0.05	0.1	0.5
Ultrapure water	0.005	0.01	0.05
Mean	0.0027	0.0069	0.041
SD	0.00017	0.00043	0.0043
Decrease	45%	31%	19%

The decrease ratio was represented by percent comparing the decreased amount with the F ion concentration (0.05, 0.1, 0.5 ppm) in ultrapure water. Statistically significant at 5%.
Abbreviations: F: Fluoride; SD: Standard Deviation

ultrapure water with the same amount of added F. On the other hand, the ratio of free F ion concentrations in artificial saliva decreased after adding low-concentration F. This indicates the presence of a synergistic action between protein and calcium when binding with added F in solution. Further research is needed to clarify the changes in F added in solution with protein and in infants' saliva for proper F administration in children.

ACKNOWLEDGEMENTS

This work was supported by JSPS KAKENHI Grant Number 23792452 for Terumi Iwasaki, JSPS KAKENHI Grant Number 23593047 and 15K11375 for Yoshimori Uchikawa in Japan.

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Cite this article

Iwasaki T, Uchikawa Y (2016) Changes in Salivary Free Fluoride Ion Concentration after Adding Low-Concentration Fluoride. JSM Dent 4(2): 1060.