Journal of Oral Science, Vol. 45, No. 4, 193-199, 2003

Original

Fluoride availability and stability of Japanese dentifrices

Lina Naomi Hashizume[§], Ynara Bosco de Oliveira Lima[†], Yoko Kawaguchi[‡] and Jaime Aparecido Cury[†]

[§]Department of Preventive and Social Dentistry, Faculty of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[†]Laboratory of Oral Biochemistry, Faculty of Dentistry of Piracicaba, State University of Campinas, Piracicaba, SP, Brazil
[‡]Department of Oral Health Promotion, Graduate School, Tokyo Medical and Dental University, Tokyo 113-8549

(Received 14 May and accepted 1 October 2003)

Abstract: The decline of dental caries in the industrialized countries can be attributed to widespread use of fluorides. The Japanese market share of dentifrices containing fluorides has increased from 12% in 1985 to 77% in 2000. For a fluoride dentifrice to be effective in the control of dental caries, an adequate concentration of the fluoride must be soluble. Several Japanese fluoride dentifrices have in their formulations calcium phosphate as an abrasive, which may react with fluoride. This study was designed to evaluate the availability and stability of fluoride in the most consumed dentifrices in Japan. The analyses were made when the dentifrices were purchased (fresh samples) and after one year of storage (aged samples) at room temperature (21.8 ± 3.6°C). Total fluoride and soluble fluoride was determined using an ion specific electrode. All dentifrices showed similar concentrations of total fluoride in fresh and aged samples in accordance with the Japanese Legislation (content of less than 1,000 ppm F). Some dentifrices, with dicalcium phosphate as abrasive, showed decreasing concentrations of total soluble fluoride and increasing amounts of insoluble fluoride over time. Although most of the Japanese fluoridated dentifrices evaluated in this study contain unstable fluoride, they were found to have sufficient concentrations of soluble fluoride to be effective in preventing dental caries. (J Oral Sci. 45, 193-199, 2003)

Key words: dentifrice; fluoride; caries prevention; stability.

Correspondence to Dr. Lina Naomi Hashizume, Department of Preventive and Social Dentistry, Faculty of Dentistry, Federal University of Rio Grande do Sul, Rua Ramiro Barcelos, 2492 Porto Alegre, RS, Brazil 90035-003
Tel: +55-51-3316-5193
Fax: +55-51-3316-5002
E-mail: lhashizume@yahoo.com

Introduction

The prevalence of dental caries in developed countries has declined over the past several decades (1). Several studies are in agreement that the main reason for the reduction of caries is the greater availability of fluoride in the oral environment, particularly the increasing use of fluoridated dentifrice over the last 25 years (2,3).

In most Western industrialized countries, the percentage of fluoridated dentifrices of all dentifrice sales is above 90%, with an upward trend (4). However, in order for the dentifrice to be efficient, it is important that the fluoride is soluble in the formulation and it is regularly present in the oral cavity, so it can interfere with the phenomena of enamel-dentine demineralization and remineralization (5).

The Japanese market share of dentifrices containing fluorides, such as sodium monofluorphosphate (MFP) or sodium fluoride, has increased from 12% in 1985 to 77% in 2000 (6). Until 1994, the market share of fluoridated toothpastes was only 46%, but after the adding of fluoride into the two most popular Japanese toothpastes (White & White® and Dentor®; Lion Company), a sudden increase in this share was verified. Several Japanese fluoride dentifrices have in their formulations dicalcium phosphate as an abrasive, which may react with fluoride ions released from MFP.

The Japanese Legislation establishes the maximum fluoride content of 1000 ppm F that is permitted in a dentifrice (7). However, the concentration of fluoride contained in the dentifrice is not indicated on the package.

For a fluoridated dentifrice to be effective in controlling dental caries, an adequate concentration of fluoride must be soluble (as fluoride ion or MFP). The soluble forms of fluoride are able to interfere with the dynamics of the caries process, reducing the demineralization and activating the remineralization of dentine and enamel. Some forms of fluoride may link to the abrasive contained in the dentifrice formulation. Thus, the total concentration of fluoride is not the concentration of the soluble and active fluoride contained in the toothpaste.

Rules and standards concerning the activity and stability of fluoride in toothpastes have been published. The US Food and Drug Administration requires that the soluble fluoride ion (F^-) for NaF toothpastes and the soluble F ions (F^- plus PO_3F^{2-}) for MFP toothpastes shall be not less than 60% of the total fluoride content. The Standards Association of Australia and the European Community have produced rules for the minimum amount of soluble fluoride that must be present in fluoride toothpaste throughout its shelf life. These rules require that at least 60% of the total fluoride content be present as a soluble ion either as F^- or PO_3F^{2-}. The latter ion is rapidly hydrolyzed in the mouth to provide F^-. However, some hydrolysis occurs slowly from the time of manufacture and combines with abrasives or other toothpaste constituents to form insoluble compounds so that the total soluble fluoride concentration falls with time (8). Futhermore, with age there is a considerable loss in the soluble fluoride concentration found in dentifrice (9).

The minimum requirement for the anti-caries effect of a dentifrice is based on the available and stable fluoride in the formulation. However, the Japanese legislation only establishes the maximum fluoride content (0.10%), without specifying quality. No studies about the availability and stability of fluoride in Japanese dentifrices were found in the literature, justifying this study.

The aim of the present study was to evaluate the availability and stability of fluoride in all the forms (i.e. total fluoride, total soluble fluoride, ion fluoride, MFP and insoluble fluoride) in the most consumed dentifrices in Japan.

Materials and Methods

Preparation of the samples

Five dentifrices, the most consumed on the Japanese market, were evaluated (Table 1). Three tubes of each brand were purchased from different stores from the Tokyo area. Their packages indicated the abrasive used, salt of fluoride present but not its concentration. Information about fluoride concentration was obtained from the Japan Dentifrice Manufacturers' Association (10). The samples were codified with letters A, B, C, D and E allowing a blind

Table 1 Information on each dentifrice analyzed in this study

Commercial brand (manufacturer)	Code	Fluoridated agent	Fluoride content expected**	Abrasive agent	Lot Number
White and White (Lion)	A	MFP*	960 µg F/g	Dibasic calcium phosphate dihydrate and alumina	W1287 W1307 W1264
Gum Dental Paste (Sun Star)	B	MFP	924 µg F/g	Dibasic calcium phosphate dihydrate	627J 212L 726N
PC Clinica (Lion)	C	MFP	960 µg F/g	Alumina	A1197 A1165 A1181
Clear Clean (Kao)	D	MFP	883 µg F/g	Silica	61621 61611 31871
Dentor (Lion)	E	MFP	960 µg F/g	Dibasic calcium phosphate dihydrate	A1126 A1115 W1088

* MFP = monofluorphosphate

** The fluoride content was not specified on the package, these data were obtained from the Report of the Japan Dentifrice Manufacturers' Association.

analysis. The initial analyses were carried out at the time of acquisition (fresh samples). Then, the tubes of the dentifrices were packed into a box and stored on a shelf of our laboratory for one year. The room temperature was measured every day for this period and the average (Mean ± SD) obtained was 21.8 ± 3.6°C. After this storage period (aged samples), final analyses were done. This study was conducted from October 2001 to October 2002.

Determination of Total Fluoride

The total fluoride (TF) represents the sum of the total soluble fluoride concentrations plus the insoluble fluoride concentrations. Insoluble fluoride represents the fluoride inactivated by the abrasive of the dentifrice. TF was determined with an ion specific electrode after diffusion with hexamethyldisiloxane (HMDS), because most of the dentifrices analyzed contained the abrasive calcium and aluminum that interferes with the direct analysis (11). The method described by Taves was used (12). A shortened plastic cap of 17 × 100 mm (Falcon 2030; Becton, Dickinson and Co, Franklin Lakes, USA) was fixed with petroleum jelly (Vaseline) in the center of a petri dish of 60 × 15 mm (Falcon 1007; Becton, Dickinson and Co, Franklin Lakes, USA). The dentifrice was weighed (± 0.01 mg) directly in the bottom of the petri dish and 3 ml of distilled and deionized water (DDW) was added to it. A volume of 0.1 ml of 1.65 N NaOH was placed in the cap. The petri dish was closed, and the cap was sealed with Vaseline. A volume of 1.0 ml of H_2SO_4 saturated with HMDS was added to the mixture (dentifrice-H_2O) through a hole made in the cap. The hole was quickly sealed with Vaseline and covered with a small piece of parafilm. HMDS enhances the rate of diffusion (separation) of fluoride by the formation of trimethylfluorosilane (TMFS), which is highly volatile and hydrophobic and thus escapes from the acidified sample. The diffusion process was carried out for 12 h (overnight) at room temperature with 80 rpm rotatory motion. The volatile TMFS is trapped in the NaOH and released fluoride in the solution. This solution was dried at 60°C for 2 h, by placing the cap in an oven. A volume of 0.4 ml of 0.66 N acetic acid was added to a plastic tube 17 × 100 mm (Falcon 2017; Becton, Dickinson and Co, Franklin Lakes, USA), which was closed with the cap that contained the dry sample. The tube was inverted, vortexed, opened and placed in contact with the fluoride ion specific electrode for analysis. The analyses were done in triplicate and TF represent the concentration of total soluble fluoride (TSF) plus insoluble fluoride (IS). IS represents fluoride inactivated by the dentifrice abrasive. The calibration standards were also acid-diffused.

Determination of soluble fluoride

The concentrations of TSF and as fluoride ion (F^-) were determined directly with an ion specific electrode with or without prior acid hydrolysis, respectively, according to the modified procedure described by Cury et al. (13). One hundred mg of each dentifrice was weighed (± 0.01 mg) in graduated centrifuge plastic tubes. Ten ml of DDW was added to each tube that was then vigorously shaken for 20 seconds to obtain a homogeneous suspension. The suspension was centrifuged at 3000 ×*g* for 10 minutes, the precipitated discarded and the supernatant kept for the analyses.

To determine the concentration of TSF, 0.06 ml of the supernatant was transferred to a plastic test tube and 0.190 ml of DDW was added to it. A volume of 0.25 ml of 2 M HCl was added to the tube that was heated at 45°C for 1 h for hydrolysis of fluoride bound to MFP moiety. Next, 0.5 ml of 1M NaOH and 1.0 ml of TISAB II (0.75 M acetate buffer, pH 5.0, containing 1 M NaCl and 0.4% of CDTA) were added to the tube. The determination of fluoride ions originally present in the dentifrice and that released from MFP by the acid hydrolysis was made in this tube. This solution was placed in contact with the fluoride ion specific electrode for the analysis.

To determine the concentration of F^-, 0.06 ml of the supernatant was transferred to another plastic test tube and 0.190 ml of DDW, 1.0 ml of TISAB II, 0.5 ml of 1 M NaOH and 0.25 ml of 2 M HCl were added in this sequence. The concentration of F^- was determined immediately using the electrode.

Thus, mathematically TSF = F^- + MFP and MFP = TSF - F^-. Considering that TF = TSF + IS, it is possible to calculate the percentage of fluoride inactivated (IS) by the dentifrice abrasive.

Fluoride analysis

A specific electrode for fluoride ORION 96-09 connected to an ion analyzer ORION EA 940 (Orion Research Inc., Boston, USA) was used for all the analyses. Previously, calibration was done in triplicate with fluoride standard solutions, containing 0.8, 1.6, 3.2 and 6.4 μg F/ml for total fluoride determination and 0.25, 0.5, 1.0, 2.0 and 4.0 μg F/ml for the determination of the soluble forms of fluoride present in the dentifrices. The analyses were validated using internal standards and a coefficient variation lower than 3% was considered as acceptable.

Statistical analysis

The calibration and concentrations determined were tested in the linear regression curve, using the software EXCEL (Microsoft), where a calculation program transformed the values of mV (millivolts) provided by the electrode in μg F/g for each dentifrice. The means and standard deviations were calculated with EXCEL.

Results

Tables 2 and 3 show the results (average of n = 3) of the concentrations of total fluoride and the total soluble fluoride as MFP and ion fluoride, in the fresh and aged samples, respectively. With regard to the concentrations of total fluoride, there was no significant difference between the fresh and the aged samples. The fresh samples and the samples used after a year of storage (aged) at room temperature (21.78 ± 3.56) had similar concentrations of total fluoride contained in the dentifrices. Both results are in accordance with the Japanese Legislation.

The concentrations of total soluble fluoride are shown in Fig. 1. The aged samples of dentifrices A, B, C and E showed a decrease of total soluble fluoride concentration as a result of storage time in ambient temperature. Only dentifrice D showed similar values for total soluble fluoride concentration in the fresh and aged samples.

With regard to concentration of fluoride as MFP, dentifrices B, C, D and E had reductions of 20.2%, 4.8%, 3.9% and 38.7%, respectively. Only dentifrice A had an increase (2.8%) in the concentration of MFP. There were decreases of the concentration of free fluoride (F^-) in aged samples of dentifrices A (87.5%), B (15.6%) and C (1.7%), while dentifrices D and E showed increases of 31.6% and

Table 2 Concentration (μg F/g) of total and soluble fluoride found in fresh samples of the dentifrices (Mean ± SD; n = 3)

DENTIFRICES	TOTAL FLUORIDE	SOLUBLE FLUORIDE	
		FI	MFP
A	1008.5 ± 46.3	190.7 ± 17.5	688.8 ± 66.2
B	993.5 ± 38.8	188.9 ± 10.0	665.2 ± 11.3
C	985.5 ± 25.6	82.9 ± 8.4	730.0 ± 22.5
D	942.0 ± 34.8	135.5 ± 8.0	783.2 ± 32.1
E	998.3 ± 29.6	154.3 ± 19.4	781.3 ± 49.9

FI = fluoride ion
MFP = fluoride as monofluorphosphate

Table 3 Concentration (μg F/g) of total and soluble fluoride found in aged samples of the dentifrices (Mean ± SD; n = 3)

DENTIFRICES	TOTAL FLUORIDE	SOLUBLE FLUORIDE	
		FI	MFP
A	995.3 ± 144.6	23.9 ± 1.5	708.6 ± 35.5
B	851.7 ± 40.6	159.4 ± 11.6	553.6 ± 26.4
C	969.1 ± 34.7	119.2 ± 9.5	696.4 ± 66.4
D	975.4 ± 133.7	177.0 ± 14.4	753.6 ± 33.4
E	1059.0 ± 18.9	234.1 ± 43.7	563.5 ± 79.6

FI = fluoride ion
MFP = fluoride as monofluorphosphate

51.7%, respectively, in the free fluoride concentration of aged samples.

Fig. 2 presents the percentage of insoluble fluoride contained in the fresh and aged samples of each dentifrice. In the fresh samples, the percentages of insoluble fluoride were low, varying from 2.6% to 14.0%. All the aged samples of dentifrices had increases in these percentages after one year of storage. Compared with their initial percentages of insoluble fluoride, dentifrices A, B, C, D and E had increases of 106.3%, 16.1%, 16.4%, 78.0% and 294.8%, respectively. Although dentifrice D had a 78.0% increase, the percentage of insoluble fluoride after one-year remained low (4.6%).

All dentifrices showed a tendency towards decreases in the concentration of total soluble fluoride and an increase in the percentage of insoluble fluoride after one year of storage at room temperature.

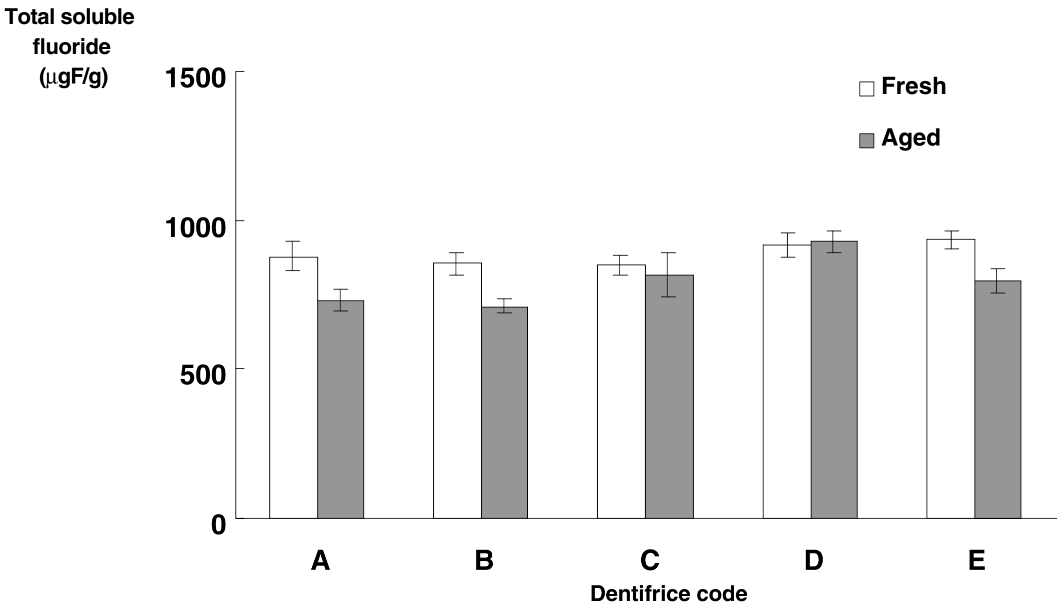

Fig. 1 Concentration (μg F/g) of total soluble fluoride found in fresh and aged samples of the dentifrices evaluated (Bars indicate SD; n = 3).

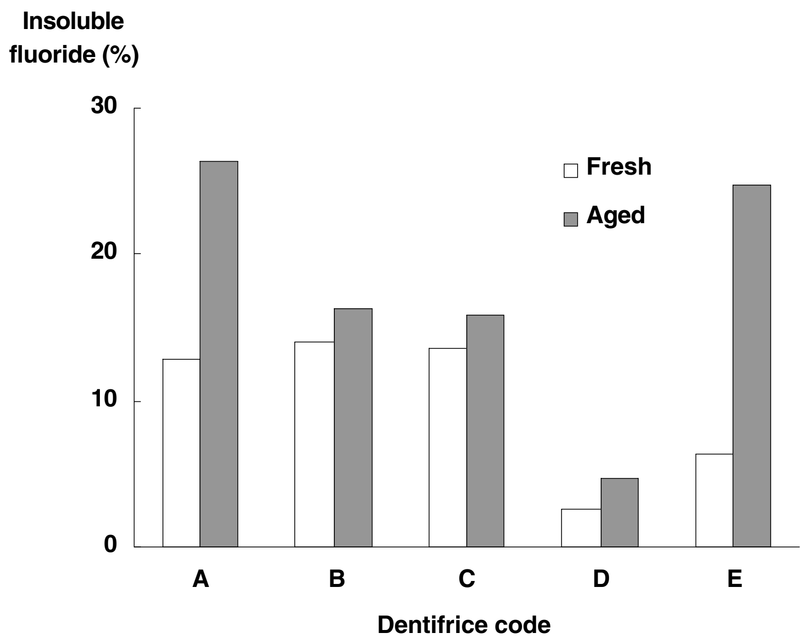

Fig. 2 Percentages of insoluble fluoride found in fresh and aged samples of the dentifrices evaluated.

Discussion

The decline of dental caries in industrialized countries can be attributed to the widespread use of fluorides, mainly in the form of fluoride dentifrices (4). Approximately 500 million people of the world's population now use fluoride dentifrice making it by far the most important fluoride delivery system (14). Tooth brushing with fluoride dentifrice has become an important public health measure in preventing caries (15).

The regular application of fluoride from toothpaste confers protection primarily by exerting a topical effect on the erupted teeth. Fluoride interacts with the plaque/tooth system in four ways: reduction of mineral solubility, inhibition of mineral dissolution, inhibition of acid production by plaque bacteria and the promotion of remineralization (16,17). However, the total fluoride contained in the dentifrice is not completely available. For the dentifrice to be effective in the prevention of caries, not only quantity but also quality of fluoride is important.

The majority of dentifrices contain either fluoride in their composition as the fluoride ion (NaF) or as MFP, which are considered active forms for controlling caries (8). Fluoride in dentifrices must be in the dentifrice in a soluble form to guarantee activity against caries (18). Depending on the formulation, part of the fluoride may be inactive and this occurs mainly in the presence of calcium (Ca^{++}) and fluoride ions (F^-). Although MFP is more stable in the presence of Ca^{++}, because fluoride is linked covalently to phosphate, it undergoes hydrolysis over time and releases F^-, which reacts with Ca^{++} to form insoluble fluoride that is inactive against caries (19).

Analyzing the five most sold Japanese fluoridated dentifrices, we verified that the total fluoride concentrations in fresh samples on the individual dentifrices were reasonably close to the values provided by the Japan Dentifrice Manufacturers' Association (10). There was no significant variation in concentration as a result of a storage time of one year. This non-variation of the total was expected, but there may be chemical changes from one form of fluoride to another as a result of product stability.

The concentration of total soluble fluoride in dentifrices A, B and E showed reductions as a result of time (Fig. 1). However, dentifrice C and D did not undergo alterations after one year. This should be attributed to the fact that these dentifrices have alumina (dentifrice C) and silica (dentifrice D) as abrasive in their formulations. Alumina and silica allow the fluoride concentration in a dentifrice to remain stable, independent of whether it is in the F- form or in MFP. On the other hand, the decrease of TSF in dentifrices A, B and E should be attributed to the fact that these dentifrices are composed of MFP, which undergoes hydrolysis over time producing F^- which turn is inactivated by the Ca^{++} of the dicalcium phosphate used as an abrasive (9,19-21). Although the data of this study have shown decreases of TSF in dentifrices A, B and E over the storage time, the minimum concentration attained has contributed to the decline of dental caries (22).

Dentifrices A, B and C had percentages of insoluble fluoride that were greater than 10%, while for dentifrices D and E the percentages were lower than 10% (Fig. 2). After one year of storage, dentifrices A and E increased their percentages to 106.3% and 294.8%, respectively. This may be explained by the hydrolysis of MFP that releases F^-, which links with the calcium of the abrasive resulting in insoluble fluoride (9,19,23).

Stability was evaluated in this study over a period of one year, however it should be emphasized that the estimated time that dentifrices stay on the shelves of the stores is less than this evaluated period. This shelf time of the dentifrices would be sufficient for no loss of soluble fluoride and indicates that dentifrices can be effective in preventing dental caries.

Over previous decades, the Japanese government and dental professionals did not encourage the population to use fluoride products and their usages were strictly limited. The high caries rate of Japanese children in the 1970's and 1980's, compared to Western industrialized countries, may be attributed to the lack in the fluoride products usage (24,25). However, the situation related to fluoride dentifrices in Japan has been changed since the beginning of the 1990's. In 1993, the market share for fluoride dentifrices was only 43%; however, this percentage reached 77% in 1999 with an upward tendency (6).

The acceptance of fluoridated dentifrices is changing in Japan, and the effect of these changes can be observed in the increase of fluoridated dentifrices in the market share and the sudden increase in people consuming these products. However, the five most consumed dentifrices evaluated in this study did not specify the fluoride content on their packages. For the Japanese consumers to make informed choices before purchase of a fluoride dentifrice, the packages should be clear in showing the fluoride concentration contained in the product.

The results of this study suggest that the five most consumed Japanese fluoridated dentifrices contain a sufficient concentration of soluble fluoride, and may be used to prevent and control dental caries.

Acknowledgments

We would like to thank Mr. Waldomiro Vieira Filho, technician of the Laboratory of Oral Biochemistry, Faculty of Dentistry of Piracicaba, State University of Campinas for help with the initial analyses.

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