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Dose-response relationship between toothpaste soluble fluoride absorbed in the gastrointestinal tract and saliva fluoride secretion

Deborah Rackel Caldas da Rocha[1], Antônio Pedro Ricomini Filho[1], Cinthia Pereira Machado Tabchoury[1], Jaime Aparecido Cury[1]*

[1] Piracicaba Dental School, University of Campinas, Piracicaba, SP, Brazil.

Aim: This study aimed to evaluate if there is a dose-response relationship between toothpaste chemically soluble fluoride absorbed in the gastrointestinal tract and fluoride secreted by saliva, giving support to the use of saliva as surrogate for plasma fluoride. **Methods:** A 4-phase single blind study was conducted, in which 10 participants were subjected in each phase to one of the assigned treatment groups: group I: fresh sample of a $Na_2FPO_3/CaCO_3$-based toothpaste with 1,334 μg F/g of total soluble fluoride (TSF) and groups II–IV: aged samples of this toothpaste presenting TSF concentrations of 1,128, 808, and 687 μg F/g, respectively. In all phases, the participants ingested an amount of toothpaste equivalent to 70.0 μg F/Kg body weight, as total fluoride (TF). Saliva and blood samples were collected before (baseline) and up to 180 min after toothpaste ingestion as indicator of fluoride bioavailability. F concentration in saliva and blood plasma was determined with a fluoride ion-specific electrode. The areas under the curve (AUC) of F concentration versus time (AUC = ng F/mL × min) and the peaks of fluoride concentration (C_{max}) in saliva and plasma were calculated. **Results:** A significant correlation between mg of TSF ingested and the AUC ($r=0.47$; $p<0.01$), and C_{max} ($r=0.59$; $p<0.01$) in saliva was found; for TF, the correlation was not significant ($p>0.05$). In addition, the correlations between plasma and saliva fluoride concentrations were statistically significant for AUC ($r=0.55$; $p<0.01$) as for C_{max} ($r=0.68$; $p<0.01$). **Conclusion:** The findings support that saliva can be used as a systemic biomarker of bioavailable fluoride present in $Na_2FPO_3/CaCO_3$-based toothpaste.

Keywords: Fluorides. Fluorosis, dental. Toothpastes. Pediatric dentistry. Saliva.

Corresponding author:
Prof. Jaime A Cury
Piracicaba Dental School, UNICAMP
CEP 13414-903, Piracicaba, SP, Brazil
E-mail: jcury@unicamp.br

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Introduction

Fluoride toothpaste is a risk factor for dental fluorosis[1] when it is ingested during brushing, but the dose (mg F/day/kg body weight) that children are exposed has been overestimated[2] because it is based on the total fluoride (TF) concentration (soluble + insoluble) present in the toothpaste formulation, but not on the chemically soluble fluoride (TSF = F ion + FPO_3^{2-} ion) fraction[3]. Dental fluorosis is a hypomineralization of enamel[4] provoked by body circulating fluoride due to fluoride ingestion during enamel formation[5].

Blood is the central compartment responsible for the distribution of fluoride throughout the human body[6], being the main marker of fluoride absorption from gastrointestinal tract[6]. Pharmacokinetics studies showed that either parotid[7] as whole saliva[8] can be used instead of blood as an alternative biomarker of fluoride (NaF) ingestion, because there is a positive correlation between fluoride concentration in plasma and that secreted in saliva. Dental biofilm and mouth contamination by fluoride oral ingestion are limitations to use whole saliva as plasma fluoride surrogate. However, they are overcome by toothbrushing made before the experiment[8] and rinsing the mouth with purified water[9,10].

Therefore, whole saliva has been successfully used to evaluate circulating blood fluoride from toothpaste ingestion[9-12]. Roldi and Cury[11](1986) evaluated the pharmacokinetics of fluoride after ingestion of six toothpastes presenting different formulations and found a high correlation (0.90) between the peak of fluoride concentration in blood and the corresponding one in whole saliva. Four years later, Drummond et al.[12](1990) compared the areas under the curves of salivary fluoride concentration after the ingestion of toothpastes and suggested that whole saliva could be an acceptable non-invasive technique to monitor systemic fluoride absorption. More recently, Falcão et al.[10](2013) also used whole saliva to show that the amount of fluoride absorbed in gastrointestinal tract depends on how much fluoride is chemically soluble in the formulation. They compared two toothpastes, one NaF/Silica-based and the other $Na_2FPO_3/CaCO_3$-based, presenting respectively, all fluoride soluble (100%) or part of it (60-80%). In addition to these specific studies comparing different commercial toothpastes formulations, Cury et al.[9] (2005) were able to differentiate the effect of fluoride concentration (0, 550 and 1100 ppm F) of NaF/SiO_2-based toothpaste on fluoride absorption. Furthermore, they showed that food in stomach reduces fluoride absorption by toothpaste ingestion, supporting the use of whole saliva as a surrogate for blood fluoride concentration to estimate the effect of substances on fluoride gastric absorption[13].

Although Roldi and Cury[11] (1986), Drumond et al.[12] (1990), and Falcão et al.[10] (2013) suggest that instead of blood, whole saliva can be used to estimate how much fluoride from toothpaste ingestion is absorbed, all of these studies compared distinct toothpaste formulations that differed in chemically soluble fluoride concentrations. To discard possible confounding factors, in the present study we used the same $Na_2FPO_3/CaCO_3$-based toothpaste formulation with 1,450 ppm F of total fluoride, but different concentrations of soluble fluoride. If whole saliva is able to differentiate how

much of the total fluoride of a toothpaste formulation is bioavailable to be absorbed in gastrointestinal tract, as shown for blood[3], a dose-response effect might also be found for saliva. If this dose-response effect is proven, saliva will indeed be able to replace blood to estimate the fluoride bioavailability of toothpastes.

Thus, the aim of the present study was to evaluate if there is a dose-response relationship between the amount (mg) of total soluble fluoride (TSF) ingested from toothpaste and the concentration of fluoride in the whole secreted saliva.

Materials and Methods

This study was approved by the Research Ethics Committee of the Piracicaba Dental School, University of Campinas (protocol number. 06195918.0.0000.5418), conducted according to the Helsinki declaration guidelines, and registered as a clinical trial [Re-BEC, ensaiosclinicos.gov.br/rg/RBR-8tkmr7]. The volunteers signed an informed consent to participate in the study.

Experimental Design and Participants

The study was carried out in Piracicaba, São Paulo, Brazil, a city with optimally fluoridated public water supply (0.6–0.8 mg F/L). Ten volunteers (5 men and 5 women) were included in the study, aged between 23 and 30 years old, weighing between 55 and 101 kg of body weight (mean of 69.5 ± 12.5 kg), presenting good general health (they reported not having gastric and/or renal disorders that could affect the fluoride absorption and excretion processes). The study was single blind (with respect to the volunteer) and consisted of 4 experimental phases, with the following treatments: group I: fresh samples of Sorriso Dentes Brancos® ($Na_2FPO_3/CaCO_3$, 1,450 μg F/g of TF and 1,334 μg F/g of TSF; Colgate-Palmolive brand; expiration date June/2021; purchased, analyzed and used in August/2019) and groups II–IV: aged samples of Sorriso Dentes Brancos® presenting TSF (F ion + FPO_3^{2-} ion) concentrations of 1,128, 808, and 687 μg F/g (21, 44, and 54% of insoluble fluoride [InsF], respectively). In each phase, all volunteers were subjected to one of the treatments that were tested in the following sequence: I, II, III, and IV. The volunteers ingested an amount of toothpaste equivalent to 70.0 μg F/kg body weight (upper limit dose for safe fluoride intake in terms of dental fluorosis)[14], as TF. A lead-in and washout periods of at least 3 days were applied[15]. Blood and unstimulated whole saliva samples were collected before (baseline) and up to 180 min after toothpaste ingestion as indicators of fluoride bioavailability. Fluoride concentration in plasma and saliva was determined with a fluoride ion-specific electrode (F-ISE) (Orion 96-09; Orion Research Inc., Boston, MA, USA), coupled to a VersaStar ion analyzer (Thermo Scientific Orion). The areas under the curve (AUC) of F concentration versus time (AUC = ng F/mL × min) and the peaks of fluoride concentration (C_{max}) in saliva and blood plasma were calculated. The dose of 0.070 mg F/Kg in terms of TF ingested was calculated based on the TF found in the toothpaste and on the body weight of each volunteer. The amount (mg) of F ingested as TSF was calculated based on the TSF concentration of the toothpaste. The hypothesis tested was that the concentration of soluble fluoride (μg TSF/g) found in $Na_2FPO_3/CaCO_3$-based toothpaste, but not TF, would be an indicator of saliva fluoride secretion. Correlation tests between the

variables tested, and between plasma and saliva were made to give statistical support to the data.

Toothpastes Accelerated Aging and Fluoride Determination

The protocol to obtain the toothpaste Sorriso Dentes Brancos® ($Na_2FPO_3/CaCO_3$, 1,450 μg F/g of TF; 1,334 μg F/g of TSF [7% InsF]), presenting different concentrations of TSF is described elsewhere[3]. The fluoride determination in toothpastes was made according to the validated protocol described by Cury et al.[16] (2010).

Volunteers' Sample Size Determination

The sample size calculation was performed as described by Caldas da Rocha et al.[3] (2022) and the result indicated that 9 volunteers would allow a power of 90% (α = 5%) to differentiate the effect of toothpastes containing 20% and 40% of InsF on the AUC of the salivary F concentration versus time. A 10% increase in sample size was considered due to the possibility of eventual losses during the study.

In vivo Study

The experiment was always conducted in the morning and after overnight fasting. In each phase, prior to the toothpaste suspension ingestion, the volunteers brushed their teeth with no fluoride toothpaste. After that, the volunteers ingested an amount of toothpaste equivalent to 70.0 μg F/kg body weight, as TF, suspended in purified water. After the toothpaste ingestion, three mouthwashes with purified water, followed by swallowing, were made to ensure complete ingestion of the toothpaste and to decrease salivary fluoride contamination by the slurry that remains in the oral cavity[9,10]. Unstimulated whole saliva samples were collected before starting the experiment (baseline data) and after 15, 30, 45, 60, 120, and 180 min of ingestion, as an indicator of systemically bioavailable fluoride.

Blood collection is described elsewhere (Caldas da Rocha et al.[3],2022). Unstimulated whole saliva was collected during 2 min. The volunteers were seated with their heads leaning forwards and asked to spit into pre-weighted plastic vials. Samples were frozen and centrifuged at 16,000 g for 10 min before analysis to remove bacteria and epithelial cells that, being intraoral sources of fluoride, could interfere with the results. TISAB III was then added to the supernatant (1:10) just before analysis[3].

Fluoride Determination in Plasma and Saliva Samples

Fluoride determination in plasma is described elsewhere[3]. For saliva, samples buffered with TISAB III (1:10) were deposited on the surface of an oil-covered inverted F electrode with the use of a microscope and micro-reference electrode, held in a micromanipulator, the same used for determination of F in plasma. The electrode was calibrated with standard F solutions, ranging from 0.056 to 1.818 μg F/mL with 10% (v/v) TISAB III. The accuracy of plasma analyses was validated using reference standards (coefficient of variation of -1.91 of triplicates). The mean coefficient of variation for the determination of F triplicates in saliva was 3.6%. A linear regression between F concentration in the standards and mV values (r^2= 0.9997 ± 0.0002;

n=11) was constructed with Microsoft Excel software and used to calculate the F concentration (µg/mL) in each sample. The AUC for saliva fluoride concentrations × time (from baseline up to 180 min) were calculated (GraphPad Prism®). For the calculation of AUC, the F concentrations due to the treatments were subtracted from the baseline value (0 time).

Statistical Analysis

The assumptions of normality of errors and homoscedasticity were checked. The amount (mg) of TF and TSF ingested were transformed into log10 and fit the required assumptions for the analyses. The amounts of fluoride ingested (mg) as TF and TSF were compared using repeated measures ANOVA. Bonferroni's test was used as a post hoc test. The relationships between the amount (mg) of TF and TSF ingested and saliva C_{max} and saliva F AUC were evaluated by Pearson's correlation test. The relationships between the AUC of plasma and saliva, and C_{max} of plasma and saliva were also analyzed by Pearson's correlation test. The level of significance was set at 5% and analyses were performed on SPSS® Statistics 21.0 software (SPSS for Windows, version 21.0; SPSS Inc., Chicago, IL, USA).

Results and Discussion

Table 1 shows that the toothpaste used was appropriate to test the hypothesis under study because the participants were subjected to the same toothpaste formulation that does not differ in amount (mg) of TF ingested ($p>0.05$) and other ingredients, but only differed ($p<0.05$) regarding TSF.

Table 1. Mean (±SD; n=8-10) of the amount (mg) of fluoride ingested by the volunteers as TF and TSF, according to the treatments.

Treatment Groups*	mg F ingested as[&]	
	TF	TSF
I: 1,334 ppm TSF	4.87 ± 0.92[a]	4.54 ± 0.86[a]
II: 1,128 ppm TSF	4.83 ± 0.91[a]	3.71 ± 0.70[a]
III: 808 ppm TSF	4.82 ± 0.91[a]	2.60 ± 0.49[b]
IV: 687 ppm TSF	4.88 ± 0.92[a]	2.13 ± 0.40[b]

*In the toothpaste used, it was found 1,442 ppm F of TF, close to the concentration declared by the manufacturer, and the values described in the table correspond to the TSF concentration determined. [&]Distinct letters indicate statistically significant differences among the groups ($p<0.05$).

Distinct curves of fluoride secreted in saliva were found (Fig.1). The distinct curves are explained by the fact that the participants were exposed to toothpaste formulations differing on TSF concentration and not on TF. The findings are explained by the fact that the gastrointestinal absorption of fluoride from toothpaste depends on how much TF is chemically soluble[3,9,10]. The results were pharmacologically confirmed by the salivary parameters AUC and C_{max} calculated. Thus, the correlation between the

amount (mg) of F ingested and salivary fluoride concentration was statistically significant (p<0.01) for TSF (r = 0.47 and 0.59, for AUC and C_{max}, respectively), but not for TF, either for AUC (r=0.06; p= 0.73) as for C_{max} (r= 0.20; p= 0.29), as shown in the inserted graphics (a and b) in figure 1. These significant correlations evidence for the first time, to the best of our knowledge, that there is a dose-response effect between the amount (mg) of total soluble fluoride (TSF) ingested from toothpaste and the concentration of fluoride in the secreted saliva. The current data extend previous results found with toothpastes that differed not only in terms of TSF[9-12]. Moreover, our results extend the Caldas da Rocha et al.[3] (2022) findings that the concentration of TSF chemically determined in toothpaste by the Cury et al.[16] (2010) protocol can be used as an indicator of systemic fluoride bioavailability (risk of dental fluorosis).

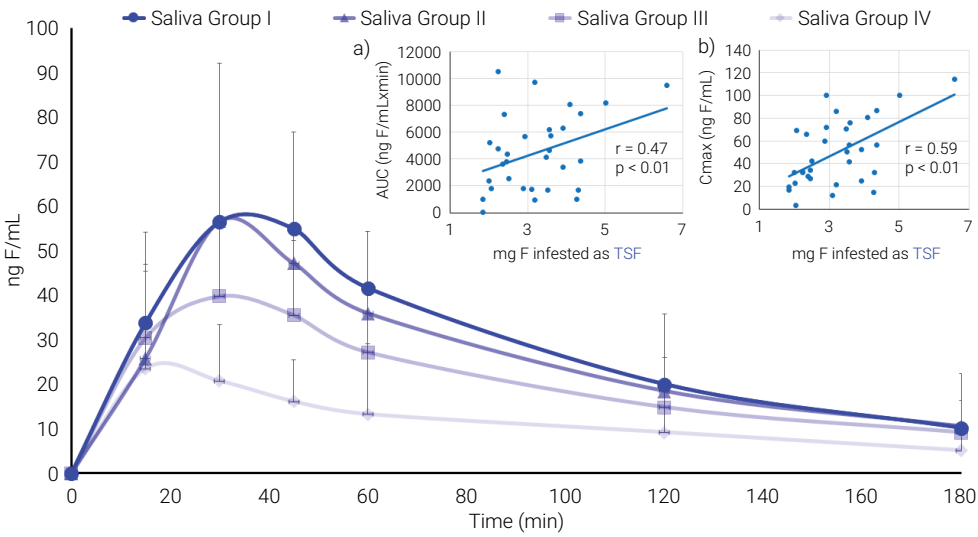

Figure 1. Mean (±SD; n = 8-10) of fluoride concentration (ng F/mL) in saliva over time (min) after the ingestion of toothpaste samples, according to treatment groups. Inserted graphics show correlations between the amount (mg) of TSF ingested and the salivary fluoride AUC (a) and C_{max} (b).

Although the pharmacological salivary parameters strongly suggest that saliva can be used as a blood marker to estimate potentially bioavailable fluoride (chemically soluble) in toothpaste, the correlations found between fluoride in saliva vs plasma (Figure 2) give additional support. Fluoride concentrations in saliva are lower than those found in plasma, what is very well known[6], but figure 2 shows that the curves are symmetric, confirming that saliva is able to mirror plasma fluoride concentration[8]. In addition, the inserted graphics (a and b) highlight that the salivary fluoride and blood are directly correlated, for AUC (r = 0.55; p < 0.01) and C_{max} (r = 0.68; p < 0.01).

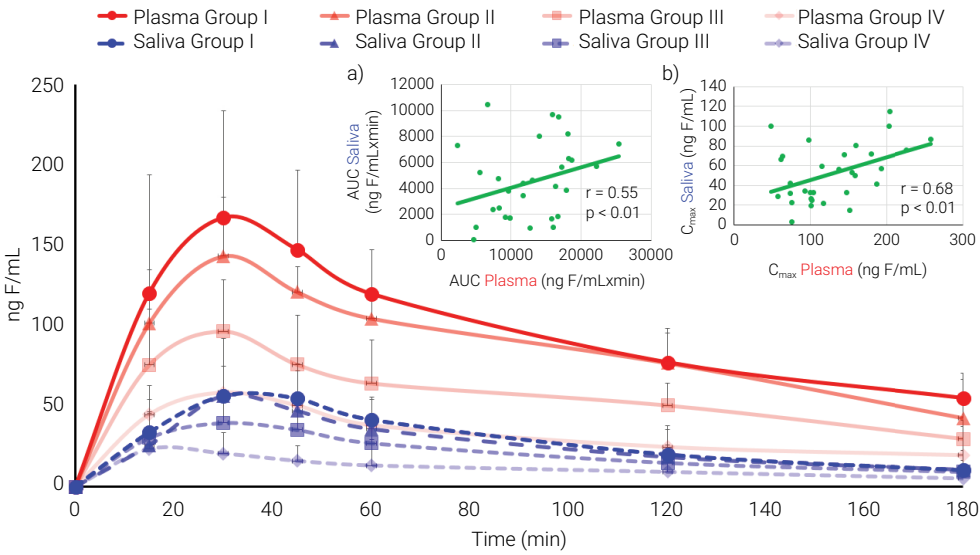

Figure 2. Mean (±SD; n = 8-10) of fluoride concentration (ng F/mL) in plasma and saliva over time (min) after the ingestion of toothpaste samples, according to treatment groups. Inserted graphics show correlations between plasma and saliva fluoride concentrations for AUC (a) and C_{max} (b).

However, the values of the correlation coefficient between AUC and C_{max} of F in saliva and the amount (mg) of TSF ingested found in the present study are lower (0.47 and 0.59) than those of plasma (0.76 and 0.86)[3]. This may be due to fluoride found in whole saliva corresponds to that secreted from blood plus traces of pre-existing F in biofilm remains and debris[8-10]. Nevertheless, saliva collection is a non-invasive procedure that is an alternative to blood, not only for fluoride analysis, but as a diagnostic tool for both oral and systemic diseases[17].

The main limitation of the present study was the fact that the results were found for Na_2FPO_3 toothpaste containing calcium carbonate ($CaCO_3$) as abrasive and there are other toothpaste formulations containing calcium in abrasive, such as calcium phosphate dihydrate ($CaH_2PO_4.2H_2O$). Although there are publications showing that the gastrointestinal absorption from this toothpaste is reduced in comparison with silica-based formulations[11,12], more studies should be done to confirm whether the dose-response results obtained for $CaCO_3$-based toothpastes would be the same for $CaH_2PO_4.2H_2O$-based toothpastes.

In conclusion, our results show that there is a dose-response effect between the amount of TSF ingested from $Na_2FPO_3/CaCO_3$-based toothpaste and the salivary fluoride pharmacokinetic parameters (AUC and C_{max}), extending the knowledge that saliva can be used as surrogate for plasma to estimate how much of total fluoride is in fact absorbed in the gastrointestinal tract.

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Data Availability

All data are available in UNICAMP repository.

Conflict of Interest

The authors have no conflicts of interest to declare.

Author Contributions

Conceived and designed the experiment: JAC Performed the experiment: DRCR Analyzed the data: DRCR, APRF, CPMT and JAC. Wrote the paper: DRCR. and JAC. Reviewed the paper: DRCR, APRF, CPMT and JAC.

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