EFFECTS OF FLUORIDE MOUTH-RINSES FOR PREVENTING DENTAL CARIES

Zita Fazakas, Stefan Hobai

REZUMAT

Introducere: Caria dentară este o boală produsă de bacterii. Progresul cariei (demineralizarea) sau inversul acestuia (remineralizarea) este determinat de echilibrul dintre factorii protectivi și patologici. În ultimii 40 de ani ionul de fluorură a jucat un rol major în reducerea cariei dentare. **Obiectiv:** Scopul studiului nostru este determinarea in vivo a efectului soluției fluorizate asupra amilazei salivare a copiilor de 9 ani. **Material și metodă:** În experimentul nostru in vivo am folosit o soluție de 0,05% NaF sub formă de soluție de clătire, aceeași soluție care este utilizată și în prevenția cariei. În studiul in vivo, efectul soluției de 0,05% NaF a fost studiat prin măsurarea activității amilazei salivare, saliva colectată la diferite intervale de timp după clătire. **Rezultate:** Studiul nostru indică faptul că amilaza salivară este inhibată de fluorură, ceea ce o face eficace în prevenția cariei dentare. **Concluzie:** În Europa Centrală și Estică, unde în continuare caria dentară are o prevalență crescută, soluția de clătire fluorizată ar fi benefică.

Cuvinte cheie: saliva umană, efectul fluorurii, soluție de clătire fluorizată, amilaza salivară

ABSTRACT

Introduction: Dental caries is a bacterially based disease. Caries progression (demineralization) or reversal (remineralization) is determined by the balance between protective and pathological factors. Fluoride ion has played a major role in dramatically reducing dental caries over the past 40 years. **Objective:** The aim of this study is to determine in vivo the effect of fluoride solution on human salivary amylase of children aged 9 years. **Material and method:** In our in vivo experiments we used a 0.05 % NaF solution as mouthrinse, the same as the one currently used in caries prevention. In the in vivo study, the effect of 0.05% NaF solutions was studied on the amylase of the human saliva collected at different periods after mouthrinsing. **Results:** Our study indicated that salivary amylase was inhibited by fluoride mouthrinses, being therefore efficacious in preventing caries. **Conclusion:** In Central and Eastern Europe where caries prevalence continues to be high and where the level of usage of topical fluorides including dentifrices will presumably remain at a low level for many years, fluoride mouth-rinses would be beneficial.

Key Words: human saliva, fluoride effects, fluoride mouth-rinses, salivary amylase

INTRODUCTION

The first sentence of the introduction to the WHO Technical Report No. 846 reads as follows: "Laboratory research suggests that fluoride is most effective in caries prevention when a low level of fluoride is constantly maintained in the oral cavity".[1,2]

Dental caries is a bacterially based disease.[3] The teeth are covered by acidogenic bacteria (mutans streptococci and lactobacilli) which constitute the dental plaque. They produce acids when metabolizing fermentable carbohydrates. The acids, such as the lactic, acetic, propionic and formic acid, can dissolve the carbonated hydroxyapatite mineral of the tooth enamel or dentin, leading to demineralization. Some studies demonstrated that the dental microbial plaque becomes acid (below pH = 5.0) within 4 to 10 minutes after sugar comes into contact with the undisturbed plaque.[4] Protective factors which include salivary calcium, phosphate and proteins, salivary flow, immunoglobulin A in saliva, fluoride in saliva and antibacterial components or agents can balance, prevent or reverse dental caries, called remineralization.[5]

Human salivary amylase is an enzyme in the oral cavity and has a pH of 6.85 - 6.9. This value decreases to acid values during the eating. Salivary amylase (α-amylase) is stable and has a maximum effect in human saliva, because its optimal pH is 6.8. Salivary amylase has been considered significant for oral health, given its intraoral activity. It is the principal digestive enzyme produced by the salivary glands and is present in

Department of Biochemistry, University of Medicine and Pharmacy, Targu Mures

Correspondence to:
Zita Fazakas, 3/1 General Ion Dumitrache Str., 540081 Targu Mures, Tel: +40-721-608118.
Email: fazzita@yahoo.com

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the parotid gland and at lower concentrations in the submandibular gland. It is a glycoprotein with a molecular weight of 62-67 kDa and plays a central role in the digestion of polysaccharides by hydrolyzing the α-1,4 glucosidal linkages of starch, glycogen, and related polysaccharides.[6] Salivary amylase is an enzyme able to bind on the bacterial surfaces and to hydrolyze starch, giving rise to products that are transformed into acids: pyruvate, lactate. On the other hand, the salivary amylase may play an important role in the colonization and metabolism of streptococcus, leading to the formation of dental plaque and caries.[7] Therefore, the salivary amylase has a carrier effect.[8]

Fluoride is a highly reactive chemical and small radius anion, able to reduce caries by three principal mechanisms: (a) inhibition of bacterial metabolism after diffusing into the bacteria as HF molecule when the plaque is acidified; (b) inhibition of de-mineralization at the crystal surfaces during an acid challenge; (c) enhancement of remineralization, thereby forming a low solubility veneer, fluoroapatite, on the remineralized crystals.[9] Fluoride occurs naturally in the Earth's crust, water, and food (fish, chicken, green tea) as the negatively charged ion, fluoride (F-). Fluoride is considered a trace element because only small amounts are present in the body (about 2.6 grams in adults), and because the daily requirement for maintaining dental health is only a few milligrams a day. About 95% of the total body fluoride is found in bones and teeth.[10] Fluoride is absorbed in the stomach and intestine. Once in the blood stream it rapidly enters the mineralized tissue (bones and developing teeth).

Nutrient interactions

Both calcium and magnesium form insoluble complexes with fluoride and are capable to significantly decrease fluoride absorption when present in the same meal. However, the absorption of fluoride in the form of monofluorophosphate (unlike sodium fluoride) is unaffected by calcium. A diet low in chloride (salt) has been found to increase fluoride retention by reducing urinary excretion of fluoride.[11]

DEFFICIENCY

Water fluoridation was the first breakthrough in the practice of preventive cariology at the community level and has remained one of the cornerstones of prevention in dentistry (water fluoride concentrations are 0.7-1.2 mg/liter or parts per million (ppm) in the USA, Canada, Ireland, Australia, Hong Kong and Singapore). Today there is general agreement that topical effects on the erupted enamel are the most important. In the fifties and sixties, fluoride tablets were widely used in Europe and in North America and helped to make the concept of caries prevention popular. The American Dental Association recommends fluoride supplements for children living in areas with suboptimal water fluoridation. From 1980 onwards, fluoride dentifrices were found to have a much greater impact and were recognized as being able to lead to a decline of caries prevalence in entire countries, and fluoride tablets gradually lost their importance. Several Latin American countries, Switzerland, France and Germany use salt fluoridation because salt fluoridation is essentially equivalent to water fluoridation.[5] Fluoride toothpastes are widespread used around the world. From 1974, the fluoride mouthrinses with a low fluoride concentration (0.05% NaF, or 230 ppm) became more popular.[12]

OBJECTIVES

The aim of this study is to determine in vivo the effect of fluoride solution on human salivary amylase of children aged 9 years. In an in vitro study, concentrations equal to or higher than 0.05 % NaF inhibited human salivary amylase.[13] Because exposure to fluoride is widespread through drinking water, toothpaste, mouthrinses, and other formulations for professional use, and being given the conflicting results in the literature, we decided to investigate the influence of fluoride on human salivary amylase by using 0.05 % NaF solutions as a mouthrinse as it is normally used in caries prevention.

MATERIALS AND METHODS

Prior to the commencement of the study the parents were informed through a leaflet regarding the aim of the study and a positive consent was requested. The protocol was approved by the Ethical Committee of the University of Medicine and Pharmacy, Targu Mures, Romania.

Subjects: Fifteen healthy volunteers (5 boys and 10 girls) aged 9 years, with no caries or gingivitis and neither prosthetic nor orthodontic apparatus. have participated to this investigation. After verbal and written information on the aim of the study, a written consent to participate was obtained from their parents.

The saliva was collected in the morning between 8.30 and 9.00 hours. Before the collection, participants were asked to rinse their mouths with 15 mL of distilled water or 0.05% NaF solution. However, before the

series of collections that followed the mouthrinses, saliva was collected for 2 min to serve as reference. The children rinsed with distilled water only, then with 0.05% NaF solution. Immediately after the rinse and then at 5, 15, and 30 min after the rinse, whole stimulated saliva was collected for 2 min.

After the collection of saliva, the pH of the collected samples was measured and the samples were centrifuged at 5,000 g for 1 hours in a VIFUG - centrifuge. The supernatant was diluted in 0.1 M Tris-buffer pH 7.0 and the resulting solution was used for determination of protein (1:10) and enzyme activity (1:50).

The pH of freshly-collected saliva samples was measured with a Consort C833 - pH meter standardized against pH 4.0 and pH 7.0 solutions.

Proteins were determined by the method of Lowry et al, using bovine serum albumin as standard. This method is based on reduction reaction of Folin reagent by the influence of the thyrosine and tryptophan from saliva. The concentration of protein was calculated by the following equation:[1]

$$[Protein]\ mg/100\ ml\ saliva = EP/ES \cdot 1.1 \cdot 103 \quad \textbf{(1)}$$

Salivary amylase activity was determined in an assay mixture containing 1.5 mL of 0.2% starch solution, 1.5 mL of 0.1 M tris buffer (pH 7.0), 0.1 mL of diluted supernatant of the centrifuged saliva and 0.4 mL of distilled water. After incubation for 60 min at 37°C, an aliquot, 0.5 mL was taken and mixed with 1 mL coopertartrate reagent. The samples were boiled 20 min than cooled off and 1 mL complex arsenic-molybdenum plus 7.5 mL bidistillated water were added. The intensity of the developed color was measured at 580 nm in a VSU 2-P spectrophotometer. The salivary amylase activity was calculated by the following equation.[2]

$$[a\text{ - }amylase]mg\ maltose/min \cdot L = EP/ES \cdot 41.66 \cdot 103 \quad \textbf{(2)}$$

RESULTS

Preliminary analysis of the data did not reveal significant differences in amylase activity between boys and girls relative to any of the parameters studied. The results were thus processed with the genders combined.

Table 1 shows the data for the in vivo study. The pH value, protein concentration, and the amylase activity were determined before and after a rinse with distilled water or with 0.05% NaF solution.

Table 1 shows the following conclusions:

1. The pH value increased immediately after rinsing with distillated water.

2. The salivary amylase activity decreased and reach a nadir in 15 minutes, then increased and didn't reach the initial value within an hour.

3. The concentration of protein increased continuously.

4. The ratio of amylase activity and protein concentration has a maximal value in saliva before rinsing with different solutions. This ratio decrease significantly in saliva obtain after mouth rinsing with distilled water. This decrease is more evident in saliva obtained after mouth rinsing with NaF solution and reached a nadir in 15 minutes, then started to increase again.

In conclusion our study shows that the effect of 50mM of fluoride mouth-rinses has a maximal effect for preventing dental caries for 15 minutes and a protective effect for 60 minutes.

DISCUSSION

Amylase activity has been the focus of many studies on the development of dental caries. There are conflicting reports on this aspect, however. Some of them show a positive or a negative relationship, whereas others show no correlation.[14] Our study shows the positive relationship between amylase activity and dental caries.

Table 1. The pH, human salivary amylase activity, protein concentration and the ratio of amylase activity and protein concentration from parafilm-stimulated whole saliva before and after mouthrinse with distilled water or 0.05 % NaF solution, collected at different times (in min) after rinsing.

Sample	pH	[α-amylase]	[protein]	[amylase]/[protein]
1. Saliva before mouth rinsing	7.43	10.709	3.367	3.487
2. Saliva after mouth rinsing with distilled water	7.9	9.805	4.083	2.716
3. Saliva after mouth rinsing with NaF solution immediately	8.062	8.548	4.352	2.242
4. Saliva after mouth rinsing with NaF solution after 5 minutes	7.82	8.158	5.781	1.639
5. Saliva after mouth rinsing with NaF solution after 15 minutes	7.766	6.741	5.956	1.275
6. Saliva after mouth rinsing with NaF solution after 30 minutes	7.661	8.373	6.331	1.888
7. Saliva after mouth rinsing with NaF solution after 60 minutes	7.44	8.181	5.807	1.435

We have determined the ratio of amylase activity and protein concentration and we have shown that our study supports the other studies from speciality literature which have demonstrated that the salivary amylase has a carrier effect and the fluoride mouth-rinses is beneficial for preventing caries.

Our conclusion justifies the above-presented data in that 50 mM of fluoride mouth rinse has a maximal effect for preventing dental caries for 15 minutes and a protective effect for 60 minutes.

CONCLUSIONS

The regular use of fluoride mouth-rinses has the following effects:

- Will remineralize the tooth structure, which has been attacked by acid;

- Fluoride coats the outside of the teeth with a layer of acid resistant fluoroapatite. This layer will wear away over time, but if the fluoride is applied daily, the layer is refreshed;

- Fluoride from mouthrinse interferes with the function of the germs in the plaque, which are responsible for metabolizing the carbohydrates into acid;

- Fluoride speed up the formation of the internal structure of the adult teeth after the eruption. When teeth first erupt into the mouth, the pulpal organs are very large. The pulp lays down more tooth structure inside its own space as one gets older. This has the overall effect of strengthening the teeth, since the teeth contain more hard, mineralized material as they mature;

- Fluoride administrated to children affects also the shape of the teeth. The difference in shape is not obvious to non-specialists. The depth of the grooves in the back teeth is reduced by fluoride. This area is where most early decay tends to develop, and the reduction in the depth of these grooves reduces the ability of the acid to penetrate through the enamel into the softer dentin underneath;

- The fluoride mouth-rinses is efficacious in preventing caries;

- In every day use a mouthrinses or/and toothpaste result in a better local effect;

- According to the WHO policy, and with regard to the high caries prevalence, systemic fluoridation seems absolutely necessary in our town, Targu-Mures.

- Based on our experience, the green tea solution containing high concentrations of fluoride (5 ppm) has an inhibition effect on amylase activity.

REFERENCES

1. World Health Organization. Fluorides and oral health. WHO Technical Report Series, Geneva, 1994, No. 846.
2. World Health Organization. The World oral health report 2003. WHO, Geneva, 2003, *http://www.who.int/oral_health*
3. Featherstone J. The science and practice of caries prevention. JADA 2000;131:887-99.
4. Stephan KW. A blind caries and fluorosis prevalence study of schoolchildren in naturally fluoridated and nonfluoridated townships of Morayshire, Scotland. Comm Dent Oral Epidemiol 2002;29:70-9.
5. Marthaler TM. Successes and drawbacks in the caries-preventive use of fluorides – lessons to be learnt from history. Or Health & Prev Dent 2003;2:129-40.
6. Hobai S, Andras I, Nemes NE, et al. Biochimie medicala, Targu-Mures, 2001, p. 96-101.
7. Scanniepo FA, Torres GI, Levine MJ. Salivary amylase promotes adhesion of oral streptococci to hydroxyapatite. J Dent Res 1995;74:1360-6.
8. Birkhed D, Skude G. Relation of amylase to starch and lycasin metabolism in human dental plaque in vitro. Scand J Dent Res 1978;86:248-58.
9. Robinson C. The effect of fluoride on the developing tooth. Car Res 2004;38:268-76.
10. Bergheim O, Barnfield WF. Lack of correlation between dental caries and salivary amylase. J Dent Res 1945;24:141-2.
11. Petersen PE, Lennon MA. Effective use of fluorides for the prevention of dental caries in the 21st century: the WHO approach. Comm Dent Oral Epidemiol 2004;32:319-21.
12. Marinho VC, Higgins JP, Logan S, et al. Fluoride mouth rinses for preventing dental caries in children and adolescents. In: The Cochrane Library, John Wiley & Sons Ltd: Chichester, 2004.
13. Nicolau J, Leite MF, Paulo S. In vitro and in vivo effects of fluoride solutions on the human salivary amylase. Fluoride 2001;34:55-60.
14. Boros I, Mozsik Gy, Keszler P. Effect of F on major salivary glands. The amylase activity, stimulated salivary flow response and cAMP levels in parotid gland of rats consuming F - via drinking water. Fluoride 1984;17:217-23.