Effect of Fluoride Mouthrinse and Toothpaste on Number of Streptococcal Colony Forming Units of Dental Plaque

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ABSTRACT

Background: Frequent topical fluoride therapy through toothpaste, mouthrinse, professional gels and solutions causes decrease in incidence, pause and repair of dental caries in the enamel. These mechanisms are done through penetration of fluoride ions (F\textsuperscript- \textsuperscript) and their replacement with hydroxyl ions (OH\textsuperscript-) of hydroxyapatite of enamel, interfere with microbial metabolism of dental plaque and bacteriostatic effect on some cariogenic bacterial strains such as streptococci. The aim of this study was to examine the effect of fluoride mouthrinse and toothpaste on the number of streptococcal colony forming units of dental plaque.

Methods: 62 children with 6-7 years old were put in two groups. Samples of dental plaque from each group were collected both before and after use of the fluoride mouthrinse and or toothpaste. The samples were cultured on blood agar to find the number of streptococcal colony forming units (CFU). The mean colony forming unit was compared inter and intra groups before and after application of Fluoride products.

Results: The streptococcal CFU of dental plaque before and after use of the mouthrinse and toothpaste respectively was (1240±1367, 1253±1341.5) and (551±716, 898±1151). Statistically, the streptococcal CFU in each group before and after use of the toothpaste and mouthrinse was significantly different.

Conclusion: The findings of this study indicated that the fluoride toothpaste and mouthrinse reduce number of streptococcal colony forming units of dental plaque. Also this reduction was not depended on level of (F\textsuperscript-) Ions, sort of vehicle of fluoride and frequent application of the fluoride mouthrinse and toothpaste.

Keywords: fluoride mouthrinse, fluoride toothpaste, colony forming unit (CFU), streptococcus

Topical fluoride therapy refers to the use of systems containing relatively high concentration of fluoride that are applied locally or topically to prevent formation of dental plaque on the erupted teeth surfaces. Thus, this term encompasses the use of fluoride rinse, dentifrices, pastes, gels, and solutions that are applied in various manners 1.

Studies of the application of professional topical fluoride products for control of dental caries were begun in the early 1940s. It has been generally accepted that the fluoride content of enamel is inversely related to the prevalence of dental caries. Keen and coworkers explored this relationship in ages of 17-22 years of old. They have found that presence of elevated fluoride in surface enamel is associated with minimal caries experience 2. Also, Depaola and coworkers have found an inverse relationship between enamel fluoride content and incidence and prevalence of dental caries 3. At the time of tooth eruption, enamel is not yet completely calcified and enamel maturation continues approximately for 2 years after eruption period.

Fluoride is derived from saliva and other sources such as calcium phosphate and promotes enamel maturation and accentuated
remineralization of white spot lesions and incipient caries. Thus, most of fluoride incorporation into developing enamel occurs during preeruptive period of enamel formation in three stages: formation, calcification, maturation and post eruptive final maturation stages.

The presence of elevated concentrations of fluoride in proximate enamel surface serves to make the tooth surfaces more resistant to development of dental caries. Fluoride ions, when substituted in hydroxyapatite crystals, for hydroxyl ions $(OH^-)$ make them more compact and stable, and more resistant to the acid dissolution, as shown is following equations:

\[ Ca_{10}(PO_4)_6(OH) + F^- \leftrightarrow 10CaF_2 + 6HPO_4^- + Ca_{10}(PO_4)_6(OH)_{2x}F_x+ + OH^- \]

b) \[ Ca_{10}(PO_4)_6(OH)_2 + F^- \leftrightarrow Ca_{10}(PO_4)_6F^+ + (OH)^- \]

The amount of formed fluoride hydroxyapatite and fluorohydroxyapatite depends on the type of fluoride compounds, age of tooth, buffering capacity of saliva and dental plaque, pH of fluoride compounds, ion concentration of fluoride and type of vehicle for application of fluoride compounds to enamel surface. In summarized, systemic and topical fluoride therapy reduces dental decay by the following three actions:

1. Conversion of hydroxyapatite to fluorohydroxyapatite, calcium fluoride and fluoroapatite causes decrease in enamel solubility, resistance to demineralization and falling to decay.
2. Decrease in acid formation (lactic acid,…) by suppression of metabolic cycle in dental bacterial plaques.
3. Accentuated remineralization of dental plaques and incipient lesions by uptake of calcium and phosphate of saliva.

Fluoride has been shown to inhibit glucose transport, carbohydrate storage, extracellular polysaccharide formation and acid formation by oral streptococci. Topical fluoride therapy may also have an antibacterial effect in the oral cavity. At the high concentration, the growth of microorganisms is inhibited. The bioavailability of fluoride depends on pH, and in low pH, fluoride is converted to HF. The HF increases permeability of bacterial membrane, affects on different enzymes in the cell membrane and inhibits ATPase in the membrane. Once acid production is inhibited, fluoride converts an unsaturated environment into more saturated or supersaturated one and directly affects the rate of dissolution and repair of the surface and subsurface enamel hydroxyapatite. A very few reports have been focused on the mechanical effects on microorganisms and pH of dental plaque.

A growing body of dental scientific knowledge suggests, however, that the caries preventive action of fluoride may also include an inhibitory effect on the oral bacterial flora involved in the initiation of dental caries such as streptococci and lactobacilli. Fluoride inhibits glycolysis of oral microorganisms by interfering with enolase enzyme and by blocking oxygenation of metabolic cycle for energy supply and reproduction and stabilizes oral ecosystem. Fluoride concentrations as low as 50PPM have been shown to interfere with bacterial metabolism. Moreover, accumulated fluoride in plaque (100 PPM) by application of APF gel (12300PPM), SnF$_2$ (8000PPM), sodium fluoride and MFP tooth paste (900-1100PPM) and sodium fluoride mouthrinse (400-900PPM) can interfere with metabolism of bacterial dental plaque, thereby supports the enamel tissue against the acid products of oral microorganisms. The presence of associated fluoride with other elements such as tin, phosphate,… has significant antibacterial activity, decreases in amount of dental plaque, changes quantity and quality of dental plaque, decreases in retentively dental plaque fell down free surface energy for friction force acquired pellicle with surface layer of hydroxyapatite in enamel. Whereas some investigators have found no change in the prevalence of cariogenic microorganisms, others have reported deductions in cariogenic streptococci. The purpose of this study is to examine the effect of sodium fluoride mouthrinse and fluoride toothpaste on quantities of streptococcal colony forming units (CFU).
Subjects and Methods
62 children with 6-7 years old were selected with simple randomized sampling and participated in this study after giving informed consent.

Inclusion criteria for selection of samples were: a) moderate oral hygiene by measurement of OHIS, b) no-use of drugs and fluoride compounds before and during the study, c) approximate dmft as 3-5, d) no history of any systemic disease and disability.

After assignment of samples, all cases were educated how to brush teeth and to rinse mouth. The teeth were brushed one time daily for 7 days under supervised with no application of fluoride toothpaste. After 7 days, we provided a sample of dental plaque on lingual surface of E tooth of all cases. The samples were transferred to bacterial culture media and sent to the microbiological laboratory of medical school of Isfahan university of medical sciences. The samples of dental plaque were prepared for culture process in two blood agar plates by microbiology technician. The culture media containing samples of dental plaques were incubated for 48 hours in 37\(^\circ\)C and the number of colony forming units (CFU) were counted and recorded in paper sheet.

The used mouth rinse and tooth paste were containing 2% sodium fluoride (500 PPM) and 1% Mono fluorophosphates (1000 PPM), respectively.

All objectives were divided into two groups: the mouthrinse and the toothpaste users. One group used the mouthrinse and the other one brushed with the toothpaste for 21 days, once daily. In the final period of study, again such as before, a sample of dental plaque was taken and transported to microbiological laboratory in order to count the streptococcal CFU. Finally the mean colony-forming units before and after application of the fluoride mouthrinse and toothpaste were analyzed and interpreted by student t-test and paired t-test.

Results
The mean streptococcal colony forming units of dental plaque before and after use of sodium fluoride mouthrinse was significantly different (p<0.001). Significant difference between mean colony forming units of dental plaque before and after use of monofluorophosphate toothpaste was also observed (p<0.05).

In comparison, the mean CFU of dental plaque before and after use of the mouthrinse and toothpaste was not significantly different (p>0.05).

<table>
<thead>
<tr>
<th>Time -type of fluoride</th>
<th>Mean CFU±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Mouthrinse</td>
<td>1240±1367</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>After mouthrinse</td>
<td>551±716</td>
<td></td>
</tr>
<tr>
<td>Before tooth paste</td>
<td>1253±1341.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>After tooth paste</td>
<td>898±1151</td>
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Discussion
In vitro and in vivo studies have shown that the inhibitory effect of fluoride on streptococcal strains is related to the time exposure, pH of the environment, concentration of fluoride ions and substantiality of fluoride compounds. The present study focused on the in vivo ef-
fecteds of two different products of fluoride tooth paste (1000PPM) and fluoride mouthrinse (500 PPM), on the quantitates of colony forming units (CFU) of lingual surface of dental plaque.

Although the effect of 1000PPM (F-) toothpaste on decrease of the streptococcal CFU, in compare with 500PPM (F-) was not significant, but each of two compounds significantly reduced the CFU. In other studies, the level of 300-600 PPM (F-) reduced viability and growth of streptococcal mutant strains 5,29.

The ability of streptococcal strains to survive, growth and colonize in dental plaques in presence of relatively high fluoride concentration corresponds with previous reports about in vitro adaptation of oral streptococci to inhibitory level of fluoride. The ability of some strains of streptococci to become refractory fluoride tolerance also may develop during prolonged topical fluoride therapy 18, 20, 30. In vivo fluoride tolerance may explain why patients on topical fluoride therapy for years, still harbor prominent plaque level of streptococcal mutants, despite presumably inhibitory concentrations of fluoride in dental plaques. The lack of caries development in these patients also may be related to the reduced cariogenicity of fluoride-resistant streptococcal mutant strains 23, 24, 25, 27, 29, 30. We recommend broad study about in vitro and in vivo carioreducibility of other fluoride compounds.

Findings of this study show that the fluoride mouthrinse and toothpaste cause reduction of streptococcal colony forming units. These findings support specific dental plaque hypothesis. More studies for confirmation of effects of other topical fluoride compounds on quality of dental plaques and potentially reduced effects of dental caries are essential.

References