Comparative evaluation of antimicrobial efficiency of marketed children’s fluoridated toothpastes at diluted concentrations against Streptococcus mutans - An in vitro study

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Abstract

Background: Dentifrices containing fluoride are known to possess anticarious benefits and are also used for enamel remineralization. A dentifrice is the best source of fluoride for children. However, there seems to be a risk of dental fluorosis as children tend to consume passable amount of fluoride from toothpastes itself. Due to the deliberate swallowing of toothpaste and the menace of fluorosis, dentifrices with lower fluoride concentrations for young children are developed and marketed.

Aim: The objective of the study is to determine and equate the efficiency of fluoridated toothpastes of children at altered or diluted levels against Streptococcus mutans.

Methodology: The preparation of the slurry was done by diluting the measured amount of toothpastes (20 g) in calculated 20 mL of sterile distilled water that gives a 1:1 (toothpaste:distilled water) concentration. Blood agar plates were made, and after solidification of culture medium, wells were made in each agar plate and all the concentrations of the two test materials, i.e., 1:1, 1:4, and 1:8 of 20 μL each were added to respective wells and were incubated at 37°C for 48 h and under strict aerobic conditions. The study was performed and repeated 10 times under sterile conditions. The zone of inhibition was measured using a digitalized Vernier caliper in millimeter.

Results: The zone of inhibition of the studied samples against the tested strain was significant at diluted levels of fluoride.

Conclusions: The fluoridated dentifrices even after having lesser fluoride concentration show antimicrobial property.

Clinical Significance: This study will help us in understanding the antimicrobial efficiency of fluoride at reduced concentrations and thereby can help curb the menace of fluorosis and nip it at the bud. By manufacturing, dentifrices contained reduced levels of fluoride.

Introduction

There has been a fundamental change in approach in the dental field that has guided the promotion of definite preventive treatment strategies, based on the current caries assessment and the risk factors associated with it.

There is a phenomenal evidence that fluoride dentifrice is an efficacious means of caries prevention, and it has been hypothesized that it is one of the principal grounds for the decline in caries.

Dentifrices containing fluoride are known to possess anticarious benefits and are also used for enamel remineralization. A dentifrice is the best source of fluoride for children. However, there seems to be a risk of dental fluorosis as children tend to consume passable amount of fluoride from toothpastes itself.
Due to the deliberate swallowing of toothpaste and the menace of fluorosis, dentifrices with lower fluoride concentrations for young children are developed and marketed. Furthermore, the Swedish National Board of Health and Welfare thereby recommended a pea-sized amount of fluoridated toothpaste to be familiarized to the children right from 1.5 years of age.[4]

Nowadays, low levels of fluorides in marketed toothpastes ranges from 250 to 500 ppm are being used by children. That being so the antimicrobial efficacy of these toothpastes yet remains uncertain.

Fluoridated dentifrices are regularly used today as an active ingredient providing fluoride to the oral cavity. Sodium fluoride (NaF), sodium monofluorophosphate (MFP), or their combinations in the range of 500–1500 ppm are commonly used commercially. Newer variations in the form of with stannous fluoride (SnF2) or amino fluoride (AmF) are available.

Furthermore, as a substitute to fluoride therapy, calcium phosphate and herbal formulations have been used in child’s dentifrices. Beltran stated that the prime intention of any fluoride therapy for children below the age of 6 years is to reap supreme effects with the insignificant peril of fluorosis.[3]

Numerous toothpastes assert to possess an antimicrobial role, but there has been paucity of studies to prove these claims on kid’s dental formulations. For that reason, we attempt to gauge the antimicrobial influence of children’s toothpastes comprising varied forms of active fluoride agents, at different concentrations against S. mutans.

Materials and Methods

In Sample A, the active ingredient present is Sodium Monofluorophosphate with 0.35% concentration (458 ppm) marketed as Cheerio Gel. [Figure 1].

In Sample B, the active ingredient present is Amine Fluoride(AmF) with 250 ppm concentration marketed as Elgydium [Figure 2].

Method

Dentifrice slurry preparation

The preparation of the slurry was done by diluting the measured amount of toothpastes (20 g) in calculated 20 mL of sterile distilled water that gives a 1:1 (toothpaste:distilled water) concentration. Moreover, successive dilutions of the sample, i.e., 1:4 and 1:8 were made[5] [Figures 3 and 4a and b].

Antimicrobial assay

A total of 60 blood agar plates were made, of which 30 plates were for CHEERIO GEL and other 30 for ELGYDIUM. A well of 3 mm in diameter and 4 mm in depth were made with the help of a sterile steel borer in each plate. After solidification of culture medium, wells were made in each agar plate and all the concentrations of the two test materials, i.e., 1:1, 1:4, and 1:8 of 20 μL each were added to respective wells and were incubated at 37°C for 48 h and under strict aerobic conditions. The study was performed and repeated 10 times under sterile conditions. The zone of inhibition was measured using a digitalized Vernier caliper in millimeter. The antimicrobial efficacy of each diluted concentrations was described as mean of diameter of inhibitory halos (in mm).
Statistical analysis
Data were collected and analyzed using the Statistical Package for the Social Sciences version 10.0 software. The results obtained were presented as mean and standard deviation and in percentage number. The level of significance was fixed at $P = 0.05$ and any value $\leq 0.05$ was considered to be statistically significant. Analysis of variance and Student's $t$-test were used to find the significance of study parameters.

Results
Zone of inhibition for both the samples at 1:1, 1:4, and 1:8 dilution was seen in Figure 5. In this study, sample B containing amine fluoride showed maximum zones of inhibition (25.91) and sample A containing 0.35% sodium MFP showed the least inhibitory halo (20.55) at 1:1 dilution. AmF (sample B) was found to be more effective than sodium MFP (sample A) at 1:1 dilutions. At a dilution of 1:8, sodium MFP i.e. (Group A) showed better results when compared to AmF i.e. (Group B). As shown in Table 1 and Graph 1.

Discussion
The potency of fluoride dentifrices in the prevention of dental caries is well reported. The increase incidence in mild dental fluorosis is a matter of great interest from the public health point of view. With appropriate intervention measures, it may be possible to reduce the trend in the prevalence of dental caries while arresting the increase in the prevalence of fluorosis. Evidently, children have many opportunities for fluoride exposures from water, foods and beverages, dietary fluoride supplements, fluoride rinses and gels, and professionally applied fluorides, as well as dentifrice. Because the use of fluoride levels in dentifrices can be adjusted, as their exposures from dentifrices often occur frequently (2 or more times per day), and they have relatively higher

<table>
<thead>
<tr>
<th>$\text{Cheerio gel (1:1)}$</th>
<th>10</th>
<th>20.55±0.575</th>
<th>22.398</th>
<th>&lt;0.001**</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Elgydium gel (1:1)}$</td>
<td>10</td>
<td>25.91±0.490</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{Cheerio gel (1:4)}$</td>
<td>10</td>
<td>17.56±0.422</td>
<td>1.699</td>
<td>0.107</td>
</tr>
<tr>
<td>$\text{Elgydium gel (1:4)}$</td>
<td>10</td>
<td>18.01±0.723</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{Cheerio gel (1:8)}$</td>
<td>10</td>
<td>11.90±0.391</td>
<td>6.707</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>$\text{Elgydium gel (1:8)}$</td>
<td>10</td>
<td>10.69±0.414</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

($P < 0.05$ - Significant*, $P < 0.001$ - Highly significant**). SD: Standard deviation, S. mutans: Streptococcus mutans
concentrations, a strong recommendations or guidelines regarding its use may be particularly effective in reducing the risk of fluorosis.

The two main risk factors that need to be addressed are, first, the fluoride intake during the period of tooth development, and second, the amount of fluoride that needs to be ingested and absorbed to elicit fluorosis.

The aim is to achieve maximum effect of fluoride in decreasing the prevalence of dental caries, while controlling the intake of fluoride, thereby reducing the risk of fluorosis.

Winter et al. conducted a double-blind study among 2-year-old children where two groups of 1055 ppm and 550 ppm fluoride levels in dentifrices were checked. No significant results were seen between the control pastes either in caries or plaque levels. Thereby, they recommended usage of low fluoride toothpaste for children. Furthermore, a similar study by Koch et al. revealed no statistically significant difference between two 1000 ppm and 250 ppm dentifrices containing (sodium MFP and NaF, respectively). Many studies have established that different fluoride dentifrices have broadly distinct antimicrobial effects which might justify the reason why some possess greater protection than others. Many current studies particularly addressed that dentifrice use in detail, with many of them found a relationship between the early uses of dentifrice and dental caries. Research has shown that Streptococcus mutans produces less amount of acid when fluoride is constantly present at lower concentration, thereby inhibiting the metabolism of carbohydrate. Furthermore, the bacterial production of adhesive polysaccharides is significantly affected.

The antimicrobial effect of child dentifrices containing sodium MFP - 0.35% and amine fluoride (second generation called Fluorinol), utilizing laboratory strains of S. mutans bacteria were examined in this study. Due to the possibility of fluorosis, children’s toothpastes have reduced the levels of fluoride by half when compared to adult toothpastes. Evidently, reducing the fluoride concentration of dentifrice may reduce its anticaries effectiveness; therefore, the prototype lower fluoride dentifrice should not only reduce risk of fluorosis but also be effective in caries prevention. In spite of these lower fluoride concentrations, the children’s toothpastes demonstrated to possess antimicrobial activity against the S. mutans strain.

The effective response of dentifrices containing AmF can be ascribed to the residues of AmF due to its amine portion that is positively charged. In this study, Fluorinol (250 ppm fluoride), a patent of Elgydium company has the property of adhering to the teeth faster and more intense than in the conventional fluoride. When compared to simple inorganic fluoride, organic fluoride has 5 times more fluoride binding in the 1st min of brushing and 12 times after the 3rd min. This property increases the resistance of teeth to acid attack from the bacteria.

One of the most commonly used active ingredients in dentifrices is sodium MFP. In this configuration, the fluoride ions are compactly arranged and need enzymatic hydrolysis to liberate fluoride ions. Consequently, a larger number of free active fluoride is obtainable on the surface of tooth, on the contrary, NaF combinations interact with the filler particles, and thereby, the availability of active fluoride is reduced.

Ritika et al. conducted a study where they compared different concentrations of sodium MFP at 0.35% and 0.38%, respectively, and found that 0.38% showed a less effective response when compared with the former. Therefore, it can be perceived that the uptake of free fluoride ion for MFP relies on the interaction with other ingredients in the dentifrice and not on the entire concentration of fluoride present. Furthermore, a study has shown the effectiveness of AmF against S. mutans at different reduced concentrations. An amalgamation of AmF with SnF2 has exhibited excellent antimicrobial results, hinders formation of plaque, and prevents inflammation.

Conclusions

It can be concluded that despite lowering the concentrations of fluoride in the child dental formulations, it has still shown to be effective against the tested strain. This acknowledgment of prophylactic worth guided to carry out investigations for better and ameliorated formulations. There is a high need for research for producing more efficacious and potent products.

References