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Antimicrobial activity of toothpastes against early childhood caries

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Abstract:
Objective: assessing antimicrobial activity of toothpastes with different ingredient composition recommended for infants and toddlers.
Materials and Methods. The study object was dental plaque of children with decompensated form of caries under the age of 3 years old. Seven toothpastes with different composition designed for early childhood were studied. Identification of detected microorganisms was carried out via mass spectrometry. Antibacterial activity was determined by agar diffusion and serial dilution methods.
Results. Toothpastes containing xylitol, lactic enzymes and ingredients of medicinal herbs exhibited the absence of a microbial growth inhibition zone. Fluoride toothpastes were active against all tested microorganisms, and the final fluoride inhibitory concentration was 0.00016-0.0025%, depending on the fluoride concentration in the toothpaste.
Conclusion. The least pronounced antimicrobial activity was detected in complex toothpastes with lactic enzymes and ingredients of medicinal herbs, as well as in samples with xylitol. Simple toothpastes with organic and inorganic fluorine compounds demonstrated the best bactericidal effect.

Keywords: early childhood caries, cariogenic microflora, toothpaste.

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Introduction

Early childhood caries is among the most common medical disorders worldwide in children in their first years of life. Chief etiological factor in the occurrence of deciduous teeth caries in infants and toddlers is the presence of cariogenic microflora (CM) in the oral cavity. The most important role in the development of caries is played by Streptococcus mutans (over 30% of the total CM) [1-5]. Other microorganisms also play an integral role in the development of caries: S. salivarius, S. sanguinis and S. mitis [6].

In the oral cavity of newborns, CM is initially absent. The emergence of cariogenic microorganisms in a newborn is due to a primary infection, the main source of which is the child's mother or other people in close contact with a child. Transmission of infection is conducted through saliva as a result of sharing tableware and noncompliance with the principles of individual hygiene. Initially, the oral cavity is inhabited by opportunistic streptococci (largely S. mitis and S. salivarius), and the formation of microflora ends by the age of four [7, 8].

To reduce the effect of CM on the hard tissues of the teeth, it is necessary to maintain a proper oral hygiene in a child from the moment the first teeth appear [9]. For this purpose, silicone fingertips, a toothbrush without toothpaste, gauze napkins (including those with xylitol) are used, and later on, toothpastes are added [1, 10]. Depending on the purpose, toothpastes are divided into two main groups: hygienic, and therapeutic and prophylactic. Hygienic toothpastes have solely a cleansing and deodorizing effect and do not contain any specific therapeutic and prophylactic additives. Therapeutic and prophylactic toothpastes are intended both for daily oral care for preventive and hygienic purposes, and for targeted prevention of dental caries and other oral diseases. The composition of such pastes includes components with antimicrobial activity (enzymes, antibacterial substances, polyhydric alcohols, trace elements), anti-inflammatory effect (extracts and infusions of medicinal plants), and remineralizing action (trace elements) [11].

Therapeutic and prophylactic toothpastes are also classified into simple and complex. Simple toothpastes have one therapeutic and prophylactic components in their composition. The subgroup of complex toothpastes includes combined and composite toothpastes. Combined toothpastes include two or more therapeutic and prophylactic components aimed at treating and preventing one type of pathology. Composite toothpastes contain components that provide the risk reduction of various pathology types.

The composition of anticaries therapeutic and prophylactic toothpastes for infants and toddlers includes fluorine compounds – both organic (amine fluoride) and inorganic (sodium fluoride, sodium monofluorophosphate), polyhydric alcohol (xylitol), extracts of medicinal plants, and lactic enzymes. The anticaries effect of these components is implemented through various mechanisms, including antimicrobial activity against oral cavity CM.

In available publications, there are data on examining the antimicrobial activity of toothpastes with different ingredient composition for adults; however, similar studies for early childhood toothpastes were not conducted.

Providing effective medical care in early childhood is extremely difficult; therefore, the formation of healthy oral hygiene habits comes to the fore, while the use of toothpastes with antimicrobial components could contribute to both active disease prevention and curative treatment, thereby implying high relevance of studying antimicrobial activity in early childhood toothpastes.

Objective – assessing antimicrobial activity of toothpastes with different ingredient composition recommended for infants and toddlers.

Materials and Methods

We conducted the analysis of various published sources on scientific research and methodology, aimed at studying the effectiveness of using different toothpastes for early childhood. On the basis of this analysis, we chose selected toothpaste samples with various antimicrobial ingredients.

Samples of toothpastes from domestic market with various components were taken included in the study (*Table 1*). We selected complex toothpastes containing various antimicrobial components (licorice extracts, lactic enzymes); and simple, single-component toothpastes with xylitol 10%, amine fluoride (0.025% and 0.05%), sodium fluoride (0.03%), and sodium monofluorophosphate (0.05%). One toothpaste from each group was included in the study.

In order to isolate the CM of the oral cavity, the material was taken from 20 patients.

The inclusion criteria were:

1. Age of patients from 0 to 3 years old (male and female);
2. CSI (caries severity index) (mean) = 4 or more (decompensated form of the course of the caries process);
3. Absence of general somatic pathology;
4. Similar diet;
5. Use of hygienic toothpastes;
6. Availability of voluntary informed consent signed by parents/legal representatives.

Table 1. Comparative characteristics of antimicrobial components of toothpaste samples for infants and toddlers

<table>
<tr><th rowspan="2">Sample ##</th><th colspan="4">Antimicrobial components</th></tr>
<tr><th>Fluorine compounds</th><th>Polyhydric alcohol</th><th>Medicinal plant extracts</th><th>Enzymes</th></tr>
<tr><td>1</td><td rowspan="3">–</td><td>–</td><td>Japanese licorice, Aloe vera</td><td>Lactoferrin, lactoperoxidase, lysozyme</td></tr>
<tr><td>2</td><td rowspan="2">10% xylitol</td><td>–</td><td rowspan="6">–</td></tr>
<tr><td>3</td><td rowspan="2">Chamomile</td></tr>
<tr><td>4</td><td>0.05% sodium monofluorophosphate</td><td rowspan="4">–</td></tr>
<tr><td>5</td><td>0.03% sodium fluoride</td><td rowspan="3">–</td></tr>
<tr><td>6</td><td>0.025% amine fluoride</td></tr>
<tr><td>7</td><td>0.05% amine fluoride</td></tr>
</table>

The study was performed in accordance with Good Clinical Practice and the principles of the Declaration of Helsinki. The study protocol was approved by the local Ethics Committee at V.I. Razumovsky Saratov State Medical University of the Russian Federation Ministry of Healthcare.

The study material was represented by dental plaque. Plaque was taken on an empty stomach, before brushing, using sterile dental instruments (tooth scalers) from the vestibular surfaces of all teeth. The dental plaque was removed from the tool with cotton swabs and placed in the accumulation medium (Hottinger broth, pH =7.2±0.2) with 5% glucose (the medium for accumulation of opportunistic aerobic and facultative anaerobic microflora), and was delivered to the bacteriological laboratory within an hour. After 3-4 hours, seeding from the accumulation medium was performed on differential diagnostic nutrient media:

1. Hottinger agar, pH (7.2±0.2) with the addition of 5% sheep blood;
2. Chocolate agar with vitamin supplements;
3. Culture media for enterococci;
4. Endo agar;
5. Sabouraud medium.

The isolated microorganisms were identified via mass spectrometry on a MALDITOF analyzer (Bruker Daltonic GmbH, Germany). Pure 24-hour cultures, grown under conditions specific to each microorganism, were used for analysis. Individual colonies were applied to a reusable metal target, covered with a matrix solution, and, after drying, mass spectrometric analysis was performed. During the MALDITOF analysis, proteins and peptides were arranged in the spectrum in accordance with the increase in their masses, creating a unique protein profile, by which strains could be reliably distinguished. Next, employing the Biotyper software, microorganisms were identified using a reference database containing over 2,500 microbial species and 7,500 strains.

A total of seven toothpastes were examined. To evaluate their antimicrobial activity, the agar diffusion method was used. Hottinger agar, pH (7.2±0.2), supplemented by 5% sheep blood (blood agar), 15 mL of each, was poured into Petri dishes, into which 0.2 mL of a suspension of an isolated microorganism (S. salivarius, S. sanguinis, S. mitis) was administered at a concentration of 1×107 mc/mL. After drying the cups, 0.1 g of samples of different toothpastes in the native state were applied. After 16-18 hours of incubation at 37 °C, the antimicrobial activity of toothpastes was assessed by the diameter of the growth inhibition zones of the studied microorganisms.

The serial dilution method was used to determine the bactericidal effect of toothpastes. For this purpose, serial dilutions of toothpastes were prepared in Hottinger broth, pH (7.2±0.2), with 5% glucose (sugar broth). The final concentration of toothpastes in the sugar broth was 200, 100, 50, 25, 1.5 and 6.25 mg/mL. Sugar broth without toothpaste was used as a control. To all test tubes with dilutions and the control tube, 0.1 mL of the studied culture suspension at a concentration of 1×107 mc/mL was added. After 24 and 48 hours, using inoculating loop #2, the content of each test tube was inoculated on a plate with blood agar. After 24 h of incubation, the minimum concentration of each toothpaste was determined, which caused no growth of microorganisms.

Results

In the course of microbiological studies of plaque in early childhood patients, the specific microflora of the oral cavity, characteristic of this age group, was identified: Streptococcus spp. (S. salivarius, S. sanguinis, S. mitis, S. anginosus, S. oralis); Staphylococcus spp. (St. aureus, St. equorum, St. sciuri); Neisseria spp. (N. subflava); Candida albicans (C. albicans); Lactobacillus spp. (L. paracasei); Rothia dentocariosa (R. dentocariosa); Actinomyces spp. S. mutans could not be identified, possibly due to the limitations of mass spectrometry method.

Other representatives of gram-positive facultative anaerobic microflora of the oral cavity, S. mitis, S. sanguinis and S. salivarius, the role of which in the development of carious lesions of the teeth is considered proven, were selected to study the antimicrobial activity of toothpastes.

Evaluation of toothpaste antimicrobial activity by agar diffusion method demonstrated that toothpastes with fluorine compounds had a pronounced activity against the investigated microorganisms (S. mitis, S. sanguinis, S. salivarius) (*Table 2*). Toothpastes with other ingredients did not demonstrate antimicrobial activity (microbial growth inhibition zone was absent).

It should be noted that the diffusion method is qualitative, since it does not allow assessing the degree of antimicrobial activity. Besides, the presence of microbial growth inhibition zone on nutrient agar medium around the studied toothpaste does not allow concluding that the samples have a bacteriostatic or bactericidal effect.

Further applied method of serial dilutions makes it possible to evaluate the bactericidal efficacy of toothpastes with various antimicrobial ingredienst depending on their concentration.

Examination of the bactericidal action of toothpastes demonstrated the effectiveness of all studied samples (*Table 3*).

The complex sample #1, in which extracts of Japanese licorice, Aloe vera extract, lactoferrin, lactoperoxidase, and lysozyme, were used as active components, had minimal activity with respect to CM. The inhibitory concentration of that toothpaste was 100–200 mg/mL.

Table 2. Results of determining the antimicrobial toothpaste properties via agar diffusion method

Sample ##	Antibacterial components	Bacterial growth inhibition zone		
		Streptococcus mitis	*S. salivarius*	*S. sanguinis*
1	Japanese licorice extract, Aloe vera extract, lactoferrin, lactoperoxidase, lysozyme	–		
2	10% xylitol, linden flower extract			
3	10% xylitol, chamomile extract			
4	0.05% sodium monofluorophosphate	+		
5	0.03% sodium fluoride			
6	0.025% amine fluoride			
7	0.05% amine fluoride			

Table 3. Results of determining the antimicrobial toothpaste properties via serial dilution method

Sample ##	Antibacterial components	Minimum inhibitory concentration of toothpaste, mg/mL Active ingredient concentration, %		
		Streptococcus mitis	*S. salivarius*	*S. sanguinis*
1	Japanese licorice extract, Aloe vera extract, lactoferrin, lactoperoxidase, lysozyme	200	100	
2	10% xylitol, linden flower extract	100 1		
3	10% xylitol, chamomile extract	100	50	50
4	0.05% sodium monofluorophosphate	50 0.0025		
5	0.03% sodium fluoride	6.25 0.00019		
6	0.025% amine fluoride	6.25 0.00016		
7	0.05% amine fluoride	6.25 0.00032		

The sample #2 with xylitol was also characterized by a low bactericidal effect, retaining that effect up to a toothpaste concentration of 100 mg/mL, which corresponded to a xylitol content of 1%. The introduction of medicinal herbs in the form of chamomile extract (sample #3) in the composition of toothpaste with xylitol double its antimicrobial microbial activity.

Single-component samples with organic and inorganic fluorine compounds were indicative of the most active toothpastes. At the same time, toothpastes with amine fluoride had a pronounced bactericidal effect against all studied microorganisms at a final toothpaste concentration of 6.25 mg/mL (the minimum taken in the study), which corresponded to a fluorine concentration of 0.00016 and 0.00032% for samples ## 6 and 7, respectively.

Sample #5 with sodium fluoride had a high bactericidal activity of the same level; the final inhibitory concentration of fluorine was 0.00019% in relation to all studied microbes.

The activity of sample #4 with monofluorophosphate was eight times lower than other fluorine-containing pastes. The bactericidal effect of the toothpaste was manifested at a fluorine concentration of 0.0025%.

The obtained data confirmed a bactericidal effect of fluorine.

Discussion

The specific microflora of the oral cavity, isolated by us from the plaque of children, is the main etiological factor in the occurrence of caries [1-3]. S. mutans is considered the most aggressive representative; however, there are reports that S. mutans was identified in less than half of children with early caries [4].

The studied toothpastes had different compositions, which caused differences in their antimicrobial activity. The active components of sample #1 were represented by Japanese licorice extract, Aloe vera, lactoferrin, lactoperoxidase, and lysozyme. Licorice extract contains glycyrrhizin (glucoside-glycyrrhizic acid), which inhibits the glycosyltransferase of microorganisms, thereby reducing plaque formation. Lactic enzymes act on the bacterial cell membrane, due to which they have a moderate bacteriostatic effect [6].

Sample #2 contained xylitol, and sample #3 contained xylitol and chamomile extract. The issue of the antibacterial effect of xylitol is debatable. It is believed that xylitol penetrates into the bacterial cell due to the phosphoenolpyruvate system of enzymes. Further, phosphorylated xylitol is converted to xylitol-5-phosphate or xylulose-5-phosphate, which is not present in the bacterial cell, so it is dephosphorylated and excreted. This metabolic cycle requires much energy resources of adenosine triphosphate, but does not replenish the energy needs of the cell, which leads to the degradation of cellular structures and the death of microorganisms [6, 12].

Samples ## 4-7 contain various fluorine compounds (monofluorophosphate, amine fluoride, sodium fluoride). The bactericidal effect of fluorine is implemented via disruption of glycolysis (fermentation of carbohydrates) and a number of metabolic processes inside the bacterial cell: e.g., transport of ions through the cell membrane, or operation of enzyme systems [6, 13, 15].

The bactericidal effect of fluorine is manifested at fairly low concentrations; however, professional academies of dentistry, national American and European dentistry societies (ADA – American Dental Association, EAPD – European Academy of Pediatric Dentistry, WHO – World Health Organization, RDA – Russian Dental Association) recommend for children under two years of age using a toothpaste with a fluoride concentration of 0.05%; and from 2 to 6 years of age, up to 0.10% [9, 13]. This is due to the fact that the main effect of fluorine is aimed at strengthening the enamel structure and enhancing its remineralization [7, 15]. However, the EAPD does not recommend using toothpastes with less than 1,000 ppm (0.1%) of fluoride. E.g., children from 6 months to 6 years old should use toothpaste with a fluoride content of 1,000 ppm (0.1%), and children over 6 years old should use 1,450 ppm (0.145%) [16]. Sodium monofluorophosphate ($Na2PO3F$) slowly dissociates into ions, the release of active fluorine occurs in up to 10 minutes. That is why toothpastes with this ingredient are ineffective. Sodium fluoride (NaF) quickly dissociates into active ions during brushing, but its effect is short-lived. Use of sodium fluoride in early childhood is preferable to sodium monofluorophosphate, since children spend less time brushing their teeth than adults. Organic fluorine compounds are more active. Amine fluoride is the most advanced fluoride ingredient today: it has a high remineralizing activity, and it creates a depot of fluorine on the enamel surface, which penetrates into the enamel over a longer time [15].

A number of authors conducted similar studies proving the antimicrobial activity of toothpastes. However, they investigated the microflora of the oral cavity as a whole but did not examine toothpastes for early childhood [17] and did not identify microbial species [18, 19].

Conclusion

Examination of the effect of toothpastes with different compositions on the main cariogenic microorganisms in the oral cavity demonstrated that complex toothpastes with lactic ferments, ingredients of medicinal herbs and xylitol exhibited the least pronounced antimicrobial activity. Simple toothpastes with organic and inorganic fluorine compounds demonstrated the strongest bactericidal effect: of those, toothpastes with amine fluoride and sodium fluoride were significantly more active than toothpastes with monofluorophosphate.

Conflict of interest: None declared.

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