Chemical Antibacterial Inhibitors used in Toothpaste

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The chemical inhibitors act according to the following objectives: to disrupt the metabolism of the bacterial plaque and biochemical mechanisms of it, or to act upon the enamel structure in order to make it more resistant to the action of the bacterial plaque. In terms of the antibacterial activity, we compared toothpastes with different antiplaque chemical inhibitors: Triclosan / Copolymer (TC) (by Colgate Total12 Advanced Clean), AminoFluorides (MPA) (by Elmex Intensive Reinigung), Stannous Fluoride/Hexameto-phosphate (SnF²) (by Blend-a-Med ProExpert) and a toothpaste based on natural extracts - echinacea and mineral salts (E) (by Parodontax). The quantitative and qualitative evaluation of the plaque after brushing with the indicated toothpaste showed a significant reduction (percentage) of the amount of bacterial plaque but also a selective reduction of bacterial species depending on the type of chemical inhibitors consisted in the toothpastes, by decreasing the forming units of colonies from the suspension (CFU/ml suspension SF).

Keywords: chemical inhibitors, triclosan, aminofluorides, stannous fluoride, bacterial dental plaque

A correct removal of plaque is performed by dental brushing and mainly by its adjuvants. The mechanical dislocation of the dental plaque achieved by dental brushing with a toothbrush is successfully completed by the chemical inhibitors present in personal and professional cleaning products. Brushing is an effective means to remove the accumulated dental plaque [1, 2, 4]. However, despite education and other preventative programs, poor brushing habits and inadequate oral hygiene are common and are reflected in the high worldwide prevalence of gingivitis and other oral conditions. Oral hygiene formulations with anti-microbial agents represent an important advance to mitigate the effects of dental plaque [1, 3, 4, 7]. A primary rationale for the inclusion of anti-microbial agents is their ability to control dental plaque and related gingivitis. These agents include chlorhexidine, triclosan, essential oils, metal salts, and other ingredients with a significant history of safe and effective use [1, 7, 9]. Their use in over-the-counter oral hygiene formulations is supported by results from a large number of clinical studies which demonstrate significant reductions in dental plaque and gingivitis [9, 12].

In the recent years, a special attention was paid to using these chemicals in various dental hygiene products, which should effectively remove the dental plaque, but which should not adversely affect the adjacent tissues and the health condition of the patient.

The antiplaque chemicals (chemical inhibitors of plaque) are circulated in the oral environment in various forms of presentations: toothpaste, mouthwash, solutions, irrigations, chewing gums, and gels. Preferably, these substances have to be always active and with predilection to act specifically in those areas of plaque deposits, but also not to be toxic to the surrounding tissues and body. These chemical inhibitors act in with consideration of the following objectives: to disrupt the plaque metabolism and the biochemical mechanisms of the plaque, or to act in the enamel structure to make it resistant to bacterial plaque.

The most common chemicals substances used in the controlling dental plaque over the time belong to the categories: antibiotics, oxygenated compounds, quaternary compounds of Amons; phenolic compounds, plant alkaloids (natural extracts), biguanides, salts, and organic or inorganic fluorides, etc. [3, 4, 7].

Experimental part

We evaluated the antibacterial effect of toothpastes that contain different chemical inhibitors upon the dental bacterial plaque. In terms of antibacterial activity, we compared toothpastes with different chemical antiplaque inhibitors: Triclosan / Copolymer (TC) (Colgate Total12 Advanced Clean), Aminofluorides (MPA) (Elmex Intensive Reinigung), Stannous Fluoride/Hexameto-phosphate (SnF²) (Blend-a-Med ProExpert) and a toothpaste based on natural extracts - echinacea and minerals (E) (Parodontax). We watched the chemical inhibitors of the toothpastes through the quantitative and qualitative evaluation of the dental plaque.

Characteristics of chemical inhibitors used in toothpastes: Aminofluorides

In 1957 Muhlemann, Schmidd and Konig published the results of the studies performed in vitro which demonstrate that certain preparations with fluorinated amines proved to be superior to inorganic fluorides in reducing the enamel solubility. That same year Irwin, Leaver and Walsh published the results of the experiments in vitro which demonstrate that aliphatic monoamines provide enamel protection from acid demineralization. These experiments led Konig and Muhlemann to test the hypothesis that the detergent action of these organic preparations may be combined with the action of fluorine, resulting in superior protection of the hard dental structures. In 1967 Muhlemann demonstrated...
the superiority of the organic fluorides compare to inorganic fluorides in caries prevention. He noticed that the fluorinated amines have a pronounced affinity towards the enamel, increasing intake of fluoride in enamel and also that they have an antienzymatic effect on the microbial activity in the plaque. His conclusions were as follows: “AmF causes the strongest enrichment of enamel in fluoride, even in low concentration, their caries-preventive action, on one hand, being due to the fluoride and to antienzymatic effect of the organic fraction in bacterial plaque, then, on the other hand, as preventing the formation of plaque due to their surface properties.” Defined as salts or addition products of hydrofluoric acid with alkaline amines, the most used AmF are:

1) substance 297 – octafluor

\[
\text{HF} \quad \text{HF} \quad \text{CH} - \text{CH} - \text{OH}
\]

\[
\text{CH}_3 - (\text{CH}_2)_{17} - \text{N} - (\text{CH}_2)_{17} - \text{N} -
\]

\[
\text{CH}_2 - \text{CH}_2 - \text{OH} \quad \text{CH}_2 - \text{CH}_2 - \text{OH}
\]

*Diffuorhidrate of bis - (hydroxyethyl) - N-hydroxy ethyl aminopropyl-octadecyl amine*

2) substance 242

\[
\text{H}
\]

\[
\text{CH}_3 -(\text{CH}_2)_{15} \text{N}^+ - \text{HF}
\]

*AmF causes the strongest enrichment of enamel in fluoride, even in low concentration, their caries-preventive action, on one hand, being due to the fluoride and to antienzymatic effect of the organic fraction in bacterial plaque, then, on the other hand, as preventing the formation of plaque due to their surface properties.*

3) substance 335 - dextafluor

\[
\text{H}
\]

\[
\text{CH}_3 - (\text{CH}_2) - \text{CH} = \text{CH} - (\text{CH}_2) - \text{CH}_2 - \text{N} - \text{HF}
\]

*AmF causes the strongest enrichment of enamel in fluoride, even in low concentration, their caries-preventive action, on one hand, being due to the fluoride and to antienzymatic effect of the organic fraction in bacterial plaque, then, on the other hand, as preventing the formation of plaque due to their surface properties.*

**Triclosan** – trichloro hydroxyphenol-ether 0.2%. (Chemical name: 2, 4, 4′-trichloro-2′-hydroxydiphenil ether) is a felnolic compound.

Through laboratory studies, JONES AND JENKINS (1991) demonstrated that the product has an undeniable bacteriostatic effect and the means by which the agent inhibits plaque. For potentiation of the antibacterial effect, triclosan was combined with a bioadhesive copolymer (lactici polyacids, glycols) (GANTREZ) in order to promote muco – grip of the agent and thus the retard effect. In addition, these copolymers provide the product release into the environment when changing the Ph [4,8,9].

**Stannous fluoride (SnF 2)**

Tin fluoride is a new generation of ingredients used in specialized toothpastes. It was originally introduced in some toothpastes in the mid 50s, and nowadays is the most performing fluoride used in dentistry, due to its multiple advantages for maintaining oral health. The complex of stannous – stannous chloride with sodium fluoride – is a recognized antimicrobial agent that helps prevent bad breath, dental plaque and gum problems. The stannous blocks the dental tubules to reduce hypersensitivity and stimulates the enamel remineralization, making it more resistant to decay. Chelating agents with a role in stabilizing of the stannum/tin complex, prevent dental stain formation and have anti-plaque characteristics by inhibiting its formation. Silica helps remove extrinsic stains [5].

**Echinacea**

It is used in the form of various preparations against colds and flu, infections and several other major and minor diseases. It contains essential oil, polysaccharides, polyacetilen, betaine, glycosides, sesquiterpenes and caryofiles, copper, iron, tannins, proteins, fatty acids and vitamins A, C and E [4,7].

**Quantitative and qualitative evaluation of the bacterial plaque**

The study is based on the quantitative comparison of the bacterial plaque by showing the components of the plaque after using the toothpastes. The bacterial plaque harvested from the tooth surfaces was inoculated on specific culture media, and then the samples were compared by percentage after brushing. Patients taken for the study were 88 with ages between 25 and 61, both smokers and non-smokers. The samples studied are compared by percentage after brushing. Patients taken for the study were 88 with ages between 25 and 61, both smokers and non-smokers. The samples studied are represented by the bacterial plaque harvested before brushing teeth with the indicated toothpaste and then after tooth brushing at 14 days.

Patients were informed about the study and its content, and they agreed to participate in its implementation over a period of two weeks, with following strictly the necessary instructions: brushing technique (altered bass), brushing 3-5 minutes, and a compulsory evening brushing.
After 24 h of incubation at 37°C in atmosphere of selective medium for isolation of staphylococci (S. aureus, Conkey for isolating Enterobacteria; Chapman medium - (Corynebacterium sp.); the lactose mediums of Type Mac staphylococci, viridans streptococci, difterimorfi media: sheep blood agar-5% for isolating the species of lysozyme which has an inhibitor effect upon bacteria.

membranes was avoided because the saliva contains with a stream of air and the contact with the mucous surfaces of molars and premolars, from the buccal and lingual sides of all teeth, avoiding contact with, gums, tongue and saliva. Before harvesting, the teeth were dried with a stream of air and the contact with the mucous membranes was avoided because the saliva contains lysozyme which has an inhibitor effect upon bacteria.

The sowings were made by using the following culture media: sheep blood agar-5% for isolating the species of staphylococci, viridans streptococci, ditterimorfi (Corynebacterium sp.); the lactose mediums of Type Mac Conkey for isolating Enterobacteria; Chapman medium - selective medium for isolation of staphylococci (S. aureus, CNS). After 24 h of incubation at 37°C in atmosphere of CO2, the following step was the identification of germs on the basis of cultural characteristics, biochemical and pathogenicity tests. Colony forming units (CFU) were calculated from dilutions yielding at least 20 colonies per plate as described previously [6].

Results and discussions

Between the two assessments, the patients were able to maintain their hygiene using the learned brushing technique, and not using other means or dental products such as mouthwash, dental floss, interdental brushes, nor chewing gum. Evaluation before the day 0, the patients abstained from tooth brushing for 24 h. The assessments and harvesting were performed on day 0 and 14 with sterile swabs which were then placed in Stuart tubes with transport medium, after that they were processed bacteriologically. The plaque was taken from the occlusal surfaces of molars and premolars, from the buccal and lingual sides of all teeth, avoiding contact with, gums, tongue and saliva.

The used toothpastes must contain in addition to detergents, abrasives, binders, humectants, flavorings, preservative and chemical antimicrobial agents for reduction of flora in the oral cavity. Thus, the toothpastes containing fluoride and active chemical inhibitors may be effective in maintaining a proper oral hygiene.

Conclusion

Sanitation breaks mechanically the bacterial plaque, leaving a clean enamel surface. Cleaning process does not destroy many bacteria, but eliminate most of them. It is not possible that one species to be removed totally. For getting a satisfactory oral hygiene it is important to consider the following: brushing technique, frequency, as well as the toothpaste used.

Toothpastes reduce significantly the quantity and quality of the dental plaque. After two weeks of brushing with the indicated toothpastes, we noticed a decrease in the amount of plaque by 42% from the initial amount of plaque (CFU/ml suspension), and also a significant reduction of the plaque quality by modifying the percentage of the identified microbial species. Each toothpaste, according to the chemical inhibitors in it, may act on certain species of micro-organisms.

From the performed study, based on the results obtained, it was revealed that Triclosan is active on Streptococcus viridans, coagulate-negative Staphylococci, Stannous Fluoride is active on Streptococcus viridans and Neisseria spp., Aminofluorides act upon Streptococcus viridans, Neisseria spp. and upon Staphylococcus coagulo-, and plant extracts are active on the Streptococcus viridans, Corynebacterium and Enterobacteria (CFU/ml suspension) (fig 1, 2).

In all the cases the dominant microbial species of the bacterial plaque is Streptococcus viridans which was significantly reduced by the chemical inhibitors of the toothpaste, but in a different way. With Triclosan the reduction of Streptococcus viridans was 61%, for tin fluoride by 40, 62% for AmF and 54% for Echinacea (fig. 3).

The studied toothpastes – Colgate Total Advanced, Blend-a-Med, Elmex and Parodontax – make a significant overall reduction of the dental plaque after brushing, but depending on the chemical inhibitors type, percentage differences are significantly changed on the microbial species (CFU/ml suspension). The quantitative and qualitative evaluation of the plaque demonstrates that the dominant bacterial species Streptococcus viridans is significantly reduced by the chemical inhibitors used in the toothpastes. It was observed that Triclosan is effective on Streptococcus viridans, Coagulate-negative Staphylococci (CNS). Stannous fluoride is effective on Streptococcus viridans and Enterobacteria. Aminofluorides as antimicrobial agent act upon Streptococci viridans,
Neisserii, while toothpaste made of plant extracts acts on Streptococci viridans and Corynebacterium spp. Due to the wide range of dental products come onto the market, it is difficult for patients to choose a toothpaste or mouthwash appropriate to the requirements. Most times, most patients want a single product to be effective on more microbial species. [5,6,13]. We can say that all the used chemical inhibitors have the capacity to reduce the most common element of the bacterial plaque, namely Streptococcus viridans, but their quantitative effect alter/is variable.

It is mandatory for the patients to realize that the bacterial plaque is the main factor in developing oro-dental diseases. Introducing the chemical inhibitors in toothpaste and using them according to the needs of patients, knowing the quality of dental plaque associated with a correct brushing technique, lead to a long term positive effect from all points of view.

Reference

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