UDC: 616.314–002–085.454.1.035.1

THE EFFECTS OF THE "LACALUT" TOOTHPASTE ON CARIOGENIC MICROORGANISMS

ЕФЕКТИТЕ НА ПАСТАТА ЗА ЗАБИ „ЛАКАЛУТ" ВРЗ КАРИОГЕНИТЕ МИКРООРГАНИЗМИ

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Abstract

The aim of this study was to establish the counts of *Streptococcus mutans* and *Lactobacillus species* in saliva before and after teeth brushing with a Lacalut dentifrice containing a combination of sodium fluoride and amine fluoride as its main components. In order to accomplish the objective, we used the Lacalut teens 8+® dentifrice, product of Arcam GmbH, Homburg, Germany. The examined group consisted of 30 healthy schoolchildren aged 10 to 11 of both sexes from an elementary school in the Centar municipality of Skopje, Republic of North Macedonia. The examinees had a good general and oral health and approximately the same DMF index. In order to obtain better precision and accuracy, the same group was used as a control group, too. The saliva samples were taken before and 20 minutes after tooth brushing, early in the morning, after at least 12 hours without oral hygiene. The counts of *Streptococcus mutans* and *Lactobacillus species* were determined using commercially available CRT bacteria® strips produced by Ivoclar-Vivadent, Liechtenstein. A significant reduction in salivary MS and LB levels was observed in all examinees. **Key words:** *antimicrobial agents, dental decay, Streptococcus mutans, Lactobacillus species.*

Апстракт

Целта на овој труд е да се утврди квантитативната застапеност на *Streptococcus mutans* и *Lactobacillus species* во плунката пред и по употребата односно четкањето на забите со паста за заби „Лакалут". Главен составен дел на оваа паста е комбинацијата на натриумфлуорид и аминофлуорид. За реализирање на поставената цел користевме паста за заби Lacalut teens 8+, производ на Arcam GmbH, Homburg, Germany. Испитуваната група беше составена од 30 испитаници на возраст од 10-11 години, од двата пола, ученици на едно основно училиште во општина Центар во Скопје, Република Македонија. Заради поголема прецизност и точност во испитувањето, оваа група беше и контролна. Испитаниците беа со добро општо и орално здравје, со слишен хигиенски режим и начин на исхрана и со приближно еднаков КЕП-индекс. Примероците на плунката беа земани пред, и 20 минути по четкањето на забите, во утринските часови, без претходно спроведена орална хигиена најмалку 12 часа. Квантитативна застапеност на *Streptococcus mutans* и *Lactobacillus species* беше одредена со комерцијално набавени стрипови CRT bacteria, Ivoclar-Vivadent, Schaan – Liechtenstein. Кај сите испитаници имаше сигнификантно намалување на бројот на кариогените микроорганизми. **Клучни зборови:** *антимикробни соединенија, дентален кариес, Streptococcus mutans, Lactobacillus species.*

Introduction

Caries is a disease that has accompanied humans since the beginning of mankind. The first assumptions regarding the etiology of dental caries date back to the new era. There have been many theories and definitions regarding its etiology, starting from the 19th century (1889) with the so-called Miller's chemical-parasitic theory, which has been scientifically proven and for the first time mentioned microorganisms as the causative agents of dental caries. Numerous investigations, from this theory, through the theories of Gottlieb, Boedecker, Martin-Schatz, Egyied and many others, up to today's most sophisticated investigations, indicate that the presence of certain bacteria in the oral cavity is perhaps the most important element in the process called dental caries or dental destruction. The second half of the 20th century has highlighted caries as a complex and multicausal disease that is caused through the interaction of 3 basic factors: the dental surface, microorganisms and the environment. Keyes and Jordan have schematically presented these three factors by three overlapping circles, one of which is the host (the surface of the tooth), the second is the trigger (the properties of the microorganisms), and the third is the environment, i.e. substrate for the microorganisms. Later on, a fourth dimension – time –

was added to this scheme. Towards the end of the last century, the classical theory prevailed according to which caries is a local, pathological destruction of hard tooth tissues caused by external causes with a progressive flow and irreversible nature, which spreads from the enamel or cement into the dentine, and later on into the pulp with the possibility of further complications in the periodontal tissues, endangering sometimes the distant tissues and even the life of the patient. The classical theory was followed by the caries-balance theory (based on the supremacy of the pathogens or of the protective factors). According to this theory, there are three main pathogenic factors: microorganisms, irregular nutrition, xerostomia, and several protective factors: saliva, fissure sealing, use of antimicrobials, fluorides and proper nutrition. The pathogenic factors, on the one hand, and the protective ones, on the other, are in a constant balance that varies and changes dynamically during the day. Depending on the change in the equilibrium, dental caries will or won't occur[1]. Despite the efforts by scientific circles to give a full definition of the onset of dental caries, there is no dental caries without microorganisms[2]. The common point of all modern understandings regarding the etiology of dental caries is that it is an infectious disease that results in the destruction of the tooth structure, and is caused by microorganisms, primarily from mutans streptococci[3,4]. Accordingly, dental caries corresponds to the description of an infectious disease with the following postulates established by Koch, and modified by Socransky[3]:

- The disease is associated with the presence of bacteria;
- The elimination of the bacteria reduces or eliminates the disease;
- The organism reacts to the bacteria;
- The bacteria also cause the disease in experimental animals;
- The bacteria possess virulent factors.

Of all so far scientifically backed up theories regarding the definition of the etiology of dental caries, we come to the most important for us, microbiological definition, according to which dental caries is defined as a localized destruction of dental tissues under the influence of microorganisms[5]. Some of them are normally present in the mouth and convert all nutrients, and especially sugars and starches, into acids[5,6,7]. The term sugar refers not only to sugar used in the household, disaccharide sucrose, but also to low-molecular carbohydrates, as well as starches[3,8,9]. Among the acids, the most important factor is the lactic acid that dissolves the mineral structure (hydroxyapatite crystals) of the teeth[10,11,12,13].

The bacteria, the acids formed by them, as well as the food and saliva residues, combine in the mouth and form a sticky substance, called plaque that adheres to the teeth. It is one of the main causes of dental caries, but also of the periodontal diseases[5,6,7,11,12,14,15,16]. The plaque is composed of gelatinous deposits of high molecular weight glucose, by which acidic bacteria are glued to the enamel. Above all, this includes Streptococcus mutans and Peptostreptococcus, possibly in association with actinomycetes[5,6,7].

Bacteria can rapidly metabolize carbohydrates in acids and their products are known under the name of acididogenic bacteria. The change in the pH value of the plaque that occurs as a result of the action of these bacteria over time is called the Stefan curve[17].

In usual clinical trials, Streptococcus mutans and Streptococcus sobrinus[3], which together with Lactobacillus species and Actinomyces are considered the most significant odontopathogens, are believed to be the main causes of dental caries[5,18]. Due to their association with dental diseases, estimating the number of mutans streptococci in the saliva and plaque can help to diagnose the caries activity[19].

Streptococcus mutans is a gram-positive bacteria forming colonies in the form of chains. It is an alpha-hemolytic blood agar and is catalase negative. A particular feature of Streptococcus mutans as a Gram+ bacterium is the production of its own antibiotics called mutacins, which inhibit the growth of other streptococci and of many other Gram+ microorganisms. Streptococcus mutans is a bacterium that is transferable, that is, it can only be transmitted through saliva[3,5]. Modern genetic techniques allow researchers to thoroughly investigate this phenomenon[20]. Studies of the microbiological composition and of the cariogenic potential of the plaque have shown that Streptococcus mutans is predominant among mutans streptococci[9,21,22,23]. It has been confirmed through immunofluorescence studies that Streptococcus mutans colonizes foremost certain specific sites within the proximal plaque. The presence of Streptococcus mutans is in very close relation to early carious lesions[24], that is, demineralization of teeth[25].

The lactobacilli constitute the second group of very important cariogenic microorganisms. Like the mutants streptococci, they produce acids that can dissolve teeth at very low pH values (acidogenicity and acido durability). They are often found in retention areas, such as fissures, cracked teeth and restoration, etc. They have been also found in the deep sections of the carious lesions where the pH value is acidic. The lactobacilli are strongly influenced by the dietary carbohydrates and sugars3. It can be concluded from certain analyzes that the dentine from the carious lesion is responsible for the sali-

vary hyper-contamination with lactobacilli[26]. The literature points to the existence of a positive correlation between the counts of lactobacilli in the saliva, plaque and dental caries[5,24,26,27,28,29,30].

The market for cosmetic products for oral hygiene is, as never before, overfilled with various products such as toothpastes, mouthwashes, dental brushes, interdental tips and brushes, etc. Many of them have established their own names and reputations over the years. Some of them have yet to penetrate the market, and some are of suspicious origin and quality. The consumers of these products find it difficult to make the right choice and decide which products to use in their everyday oral hygiene. Among the several long-established brands in the field of oral hygiene is the Lacalut toothpaste.

The name Lacalut has been derived from the main active substance, Aluminum lactate. Aluminum lactate is a lactic acid salt, which has a pronounced astringent and anti-inflammatory effect. Because of this unique effect, Lacalut is recommended primarily to people suffering from inflammatory periodontal diseases in the oral cavity, bleeding gums, and also for the prevention of caries and in the case of hypersensitive tooth enamel[31,32,33,34].

Aim of the study

The aim of this study was to obtain our own results regarding:

- The reduction of cariogenic flora, and consequently of dental caries;
- The determination of the quantitative presence of Streptococcus mutans and Lactobacillus species in the saliva before and after the use of the Lacalut toothpaste;
- The examination of the antimicrobial effect of the Lacalut toothpaste.

Material and methods

In order to accomplish our goal we used the Lacalut teens 8+ toothpaste®, a product of Arcam GmbH, Homburg, Germany.

The main components of this toothpaste are sodium fluoride (NaF) and amino fluoride. Fluorides have a strong affinity for enamel and increase the amount of fluoride in it even at low concentrations, having also an anti-enzyme effect on the microbial activity of the dental plaque. The 1400 ppm fluoride content provides double protection for young permanent teeth. The taste of lemon and spearmint (mint) in microcapsules turns teeth washing into a real feeling of freshness.

The study included 30 examinees aged 10-11 of both sexes at a primary school in the Centar municipality of Skopje, Republic of North Macedonia. For higher precision and accuracy of the tests, this group was also used as a control group. The examinees had a good general and oral health, with similar hygienic regimes and diets and with approximately equal DMF indexes. The examinees and their parents were advised not to take food for at least 12 hours before the examination. The pupils were also without any antibiotic therapy during the examination period. Saliva samples were taken before and 20 minutes after teeth brushing with the Lacalut teens 8+ toothpaste® in the morning, without no oral hygiene performed over at least 12 previous hours. In order to determine the total count of cariogenic microorganisms, the saliva was taken by spitting approximately 1-3 ml saliva in separate sterile plastic bottles intended for the purpose.

The samples for the determination of the quantitative presence of S. mutans and Lactobacillus species were taken with commercially procured CRT bacteria strips®, produced by Vivadent, Schaan, Liechtenstein, having a microbiological medium for selective isolation of S. mutans on their first side and for the isolation of Lactobacillus species on their opposite side. Thus, when a sample was taken, its planting was automatically performed. After an incubation period of 48 hours at 35-37^{O}C, the grown colonies were counted, and these counts were used to determine the approximate number of bacteria cells, using the assumption that one cell gives rise to one colony, so this number was expressed as colony forming units (CFU). When using the aforementioned strips, the colonies of S. mutans are transparent on a blue background, and the colonies of Lactobacillus species are gray-white on a green background.

Results

The determination of the quantitative presence of Streptococcus mutans and Lactobacillus species in the saliva before and after the use of the Lacalut teens 8+ ® toothpaste, as well as the reduction of the cariogenic flora, determined by CFUs before and after using the same toothpaste, are shown in Tables 1 and 2, as well as in Charts 1, 2, 3 and 4.

Three examinees had an increased value of both analyzed cariogenic microorganisms, Streptococcus mutans and Lactobacillus species, before using Lacalut teens 8+® toothpaste. (Table 1; Chart 1, 2). After teeth brushing, 6 examinees for Streptococcus mutans and 7 examinees for Lactobacillus species were not registered with growth of colonies (Table 1; Chart 1, 2). According to the dynamic index, a registered tempo of growth is 100 %. (Table 2) Chart 3.

Table 1. Number of examinees with CFUs of *Streptococcus mutans* and *Lactobacillus species* in 1 ml saliva before and after tooth brushing with Lacalut teens 8+® toothpaste

	Streptococcus mutans CFU/ml		Lactobacillus CFU/ml	
	before	after	before	after
No growth	3	6	3	7
10^2	0	3	0	1
10^3	7	10	9	13
10^4	6	5	4	7
10^5	9	6	14	2
10^6	5	0	0	0
N	**30**	**30**	**30**	**30**

Table 2. Determination of the indexes of dynamics in patients with a *Streptococcus mutans* before and after the use of the Lacalut toothpaste

	Streptococcus mutans		Lactobacillus	
	before	after	before	after
No growth	10%	20%	10%	23.30%
10^2	0%	10%	0	3.30%
10^3	23.30%	33.30 %	30%	43.30%
10^4	20%	16.70%	13.30%	23.30%
10^5	30%	20%	46.70%	6.7%
10^6	16.70 %	0%	0%	0%
N	**100%**	**100%**	**100%**	**100%**

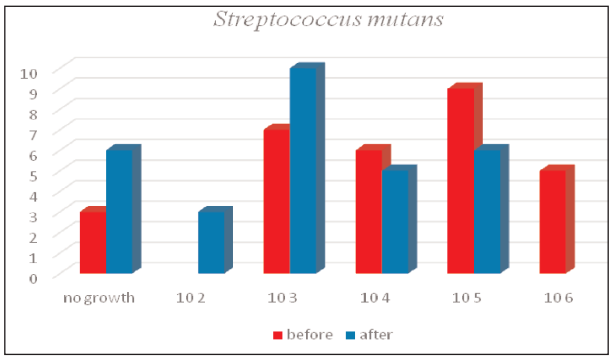

Chart 1. *Number of examinees with CFUs of Streptococcus mutans in 1 ml of saliva before and after teeth brushing with the Lacalut toothpaste*

With reference to Streptococcus mutans, the number of 103 CFUs were registered in 7 examinees before using the Lacalut teens 8+® toothpaste, and in 10 examinees after brushing the teeth with the same toothpaste. According to the dynamic index, there was a growth pace of 30% (Table 1, 2; Chart 2, 3).

In respect of the Lactobacillus species, the number of 103 CFUs were registered in 9 examinees before using

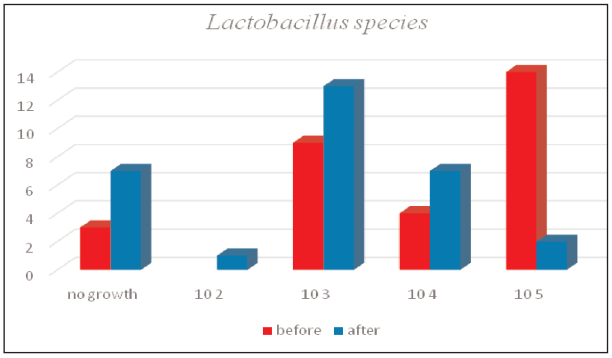

Chart 2. *Number of examinees with CFUs of Lactobacillus species in 1 ml saliva before and after teeth brushing with the Lacalut toothpaste*

Table 3. Number of examinees with logarithmic reduction factor for the Lacalut toothpaste

Log RF	Streptococcus mutans		Lactobacillus	
	Number	%	Number	%
0	8	26.7	10	33.30
1	15	50.0	12	40.0
2	4	13.30	4	13.30
3	2	6.7	3	10.0
5	1	3.3	1	3.3
N	**30**	**100.0**	**30**	**100.0**

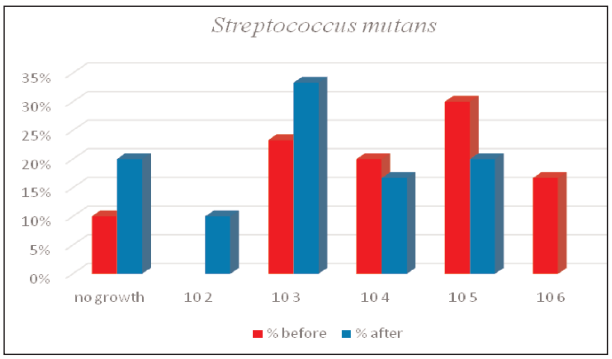

Chart 3. *Determination of the indices of dynamics in examinees with a Streptococcus mutans number registered before and after the use of the Lacalut toothpaste*

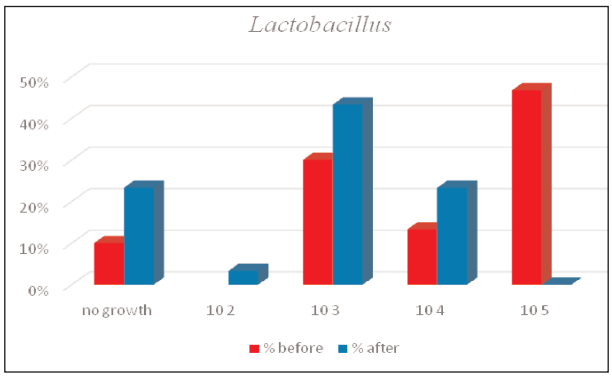

Chart 4. *Determination of the indices of dynamics in patients with Lactobacillus species number registered before and after the use of toothpaste Lacalut*

the Lacalut teens 8+® toothpaste, and in 13 examinees after brushing the teeth with the same toothpaste. According to the dynamic index, there was a growth pace of 44% (Table 1, 2; Chart 2, 4).

The total number of CFUs of 105 before using the Lacalut teens 8+® toothpaste is registered in 9 examinees for Streptococcus mutans and in 14 examinees Lactobacillus species. After tooth brushing, the number of examinees with this CFUs decreased by two for Streptococcus mutans even by 12 for Lactobacillus species. According to the dynamic index, a decline trend of 33% was found for Streptococcus mutans and 85% for Lactobacillus species (Table 2; Chart 1, 2, 3 and 4).

The number of 106 CFUs before using the Lacalut teens 8+® toothpaste, is registered only for Streptococcus mutans where 5 examinees have this number of colonies. The results have shown drastic decline after using this toothpaste where there was no subject with CFUs. (Table 1, Chart 1).

Due to the large differences obtained by determining the quantitative presence of cariogenic microorganisms before and after the use of the Lacalut teens 8+® toothpaste, ranging from 0 to > 1,000,000, the values in the following table 3 are shown logarithmically. In order to perceive the exact number of examinees in the table, in which the reduction of cariogenic microorganisms occurred, a summary is given of the number of examinees in which colonies were isolated, expressed through the logarithmic reduction factor (logRF) of 0 to > 5, specifically for Streptococcus mutans, and specifically for Lactobacillus species with logRF = logCFU before - logCFU after the use of the Lacalut teens 8+® toothpaste. The examinees with no isolated colonies were not taken into account and the total number (N) is given for the actual number of examinees who entered the analysis (Table 3)

Discussion

Over the last 20 years most papers dealing with the Lacalut toothpaste have proven its caries-inhibitory effect in all three stages of progression development of dental caries (initial, advanced and deep caries), as well as its astringent action at very small concentrations of its main active ingredients, which are: aluminum lactate, aluminum fluoride, hydroxylapatite, calcium lactate, phosphate, zinc chloride and sodium fluoride[31,32,33,35,36].

The essential active ingredient in Lacalut toothpastes (dentifrices) is aluminium lactate, a substance with good astringent properties even in low concentrations. This does not only have an immediate favourable effect on the gingiva and the oral mucosa, but also a prophylactic effect against inflammations of the gums and parodontopathies. Lacalut toothpastes have excellent cleansing properties proven in many in vitro tests and in vivo trials which are also attributed to the intensive, astringent effect of aluminium lactate[31].

Apart from aluminium lactate, Lacalut toothpastes also contain aluminium fluoride. Both fluoride, as a chemical element, and its reduced form – fluoride anion (F-), possess certain antimicrobial properties. Different concentrations of fluorides, which could bring about an inhibition of both dental plaque and oral microorganisms clear culture have been analysed. According to Tatevossijan[37], a concentration of 0.005 ml/l is capable of reducing the generation of dental plaque. Duguid and Senior[38] have investigated a number of cultures of Streptococcus sanguis, and have shown that a fluoride concentration below 0.005 ml/l has a lower effect on the investigated bacteria. The growth of bacterial cells can be reduced with concentrations between 0.01 and 0.05 ml/l, whereas it can be stopped completely with concentrations of 0.1 ml/l of fluoride. Bowden has confirmed that fluorides can kill streptococci in vitro with concentrations within the range from 0.16 to 0.31 ml/l[39].

It seemed therefore advisable to test this toothpaste for its caries-reducing effect. For this purpose a test with animals was performed. This test showed unmistakably, that the toothpaste under examination[31] inhibits dental caries in all stages of severity (initial, advanced, severe). This effect is mainly attributed to the characteristic ingredient aluminium lactate, although aluminium fluoride is also an important but not decisive component for the caries inhibiting effect[31].

G. Neuman et al.[32] concluded that fluoride ions also remain bio-available after prolonged storage times of the toothpaste, and this is an important precondition for an effective tooth-decay prophylaxis with fluoride-containing dental care products. Fluoride ions must not be inactivated by cleansing particles or other toothpaste ingre-

dients. By means of a special measuring device the content and stability of the fluoride ions in several fluoride containing toothpastes were determined, with relation to the cleansing particles (abrasives) and the fluoride compounds used in the product. It could be demonstrated that the specific combination of active ingredients aluminium fluoride complex and aluminium lactate has a very high stability of ionogenic fluoride and a completely satisfactory result was achieved, particularly in view of the used cleansing particles aluminium oxide hydrate and also the combination of aluminium-oxide hydrate and silica. Toothpastes which also contain aluminium ions, apart from the biologically available fluoride, have a particular importance for caries prophylaxis, since they do not only incorporate fluoride but also aluminium in the dental enamel. This increases the protective effect[32].

P. Riethe[33] investigated the caries-inhibiting effect of the aluminium fluoride hydroxide complex and of aluminium lactate, alone or in combined use and in different toothpastes in rats. Both aluminium and fluoride inhibit the formation of caries. The caries-inhibiting effect of both compounds totaled up in the combined use[33].

Christoph Gaasch et al.[36] compared two toothpastes with different formulations and showed that the ingredient aluminium lactate with its astringent, i.e. protein-coagulant effect leads to a desensitisation of sensitive dental necks[36].

Conclusion

From the results obtained by determining the quantitative presence of the cariogenic microorganisms Streptococcus mutans and Lactobacillus species we found that in all subjects there was a decrease in the number of colonies after the use of the Lacalut teens 8+® toothpaste.

This study is the first scientific attempt to examine the antimicrobial effect of the Lacalut teens 8+® toothpaste which provides additional mineralization and protection against cavities through an optimal concentration of organic and inorganic fluorides in its composition.

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