Effect of fluoride preparations on the activity of human salivary cathepsin C

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Abstract

Preparations containing organic and inorganic fluorine compounds are used for oral hygiene. Fluoride ions contained in these preparations display high bioactivity and can alter the environment of the mouth.

The aim of the study was to determine the effect of preparations containing aminofluorides, commonly used in oral hygiene, on the activity of salivary cathepsin C (EC 3.4.14.1). The research material included mixed saliva, collected at rest before and after the application of the following preparations: Elmex gelee, Elmex red fluid, Elmex green fluid, Fluormex rinse. The salivary pH, concentration of fluoride ions and activity of cathepsin C were determined.

Fluoride preparations inhibit the activity of cathepsin C and cause changes in human salivary pH. Saliva can serve as a diagnostic material in the examination of the environmental exposure to fluorides.

Key words: fluoride, saliva, cathepsin C.

Introduction

Regular provision of fluoride ions to the environment of the mouth promotes remineralization of early enamel lesions and inhibits demineralization and growth of cariogenic bacteria [1]. Commonly available preparations for caries prevention contain different amounts of aminofluorides and are applied in the form of mouth-rinse or gel. Aminofluorides, being organic fluorides, have a better clinical effect compared to inorganic fluorine compounds, due to specific molecular structure. The aminofluoride molecule is composed of a fluoride ion bound to the organic amine of fatty acid, which is a hydrophilic polar molecule [2]. Because of its polar structure, the fluoride ion is actively distributed and stored on tissue surface, contrary to NaF which penetrates tissues in the passive way. This is accompanied by the origin of calcium fluoride, which serves as the reserve of long-lasting release of fluoride ion to the environment of the mouth [3].

Besides, aminofluorides exert an antibacterial effect via inhibition of bacterial metabolism in dental plaque, which is a pathogenic factor in the mouth [4]. Inhibition of the activity of a number of enzymes, including the cellular ones, is one of the mechanisms of fluoride action [5].

In the inflammatory processes in the mouth, lysosomal proteolytic enzymes are released, acting as a pathogenic factor in a number of diseases. One of them is cathepsin C (dipeptidylpeptidase I), an exopeptidase, separating dipeptides from the N-end of polypeptides and proteins [6]. It has been found to have transferase properties [7].

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Material and methods

Material for analyses contained mixed saliva samples collected at rest, directly into test tubes (Z PS type, Medlab) at least 2 hours after meal from 49 healthy non-smoking and caries-free subjects, aged 19-24 (30 women and 19 men). They were divided into 4 groups, each group receiving different preparation. Saliva was collected before the preparation was applied after rinsing the mouth with distilled water and following a single use of the preparation. A pH/ionometer CPI-501 ELMETRON was used

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Table 1. **Mean values of the parameters analysed in overall research material**

	Elmex gelee (120 ppm Olaflur+Dektaflur+NaF)			**Elmex green fluid** (125 ppm Olaflur, 125 ppm KF)			**Elmex red fluid** (100 ppm Olaflur, 150 ppm NaF)			**Fluormex rinse** (200 ppm aminoF)		
	Before	After	p*	Before	After	p*	Before	After	p*	Before	After	p*
pH	7.03 ±0.57	7.35 ±0.32	0.126	6.85 ±0.36	7.23 ±0.46	0.004	6.97 ±0.37	7.10 ±0.24	0.164	7.05 ±0.03	7.23 ±0.29	0.245
[F^-] mg/dm^3	2.05 ±2.77	192.65 ±112.29	0.002**	1.63 ±1.5	44.0 ±23.34	0.002*	0.00	34.65 ±18.08	0.002*	0.00	55.57 ±15.18	0.063**
Protein mg/ml	1.07 ±0.23	1.09 ±0.21	0.821	0.37 ±0.22	0.29 ±0.12	0.072	0.66 ±0.23	0.8 ±0.18	0.002	0.82 ±0.16	0.87 ±0.2	0.686
Cathepsin C; pNA nmol/ml	138.81 ±81.87	78.46 ±59.47	0.004	127.7 ±116.2	35.0 ±35.74	0.019	77.28 ±74.22	43.89 ±34.45	0.044	66.78 ±72.73	40.26 ±28.95	0.459

* p value of Student's t-test for dependent variables
** sign test for dependent variables was used due to a lack of normal distrubution of variables

to determine salivary pH, a fluoride ionoselective electrode was employed to assess fluoride concentration, Gly-Phe-pNA substrate to detect cathepsin C (EC 3.4.14.1) [8] and Bradford's method to evaluate protein content [9]. The enzyme activity was measured by assessing the amount of released p-nitoaniline and expressed in nmol/ml [10]. The following preparations containing fluorides were used: Elmex gelee, Elmex green fluid, Elmex red fluid, Fluormex rinse. The results were subjected to statistical analysis using Statistica programme 6.0, StatSoft. Normality of distribution of the respective variables was determined with Kolmogorow-Smirnow test. To compare mean values of the respective parameters, Student's t-test was employed for dependent variables in the case of normal distribution parameters and a non-parametric sign test for dependent variables in the case of abnormal distribution parameters.

Results

Tab. 1 presents a list of mean values of the parameters after the use of aminofluoride-containing preparations. As no significant differences were noted in the parameters between women and men, gender was not the criterion.

The application of fluoride preparations caused an increase in salivary pH, which was the highest after the use of Elmex green fluid (0.38) and Elmex gelee (0.32). In all study subjects after the application, the concentration of fluoride ions increased proportionally to their levels in the preparation applied. In the case of Elmex gelee, the fluoride concentration was the highest and increased statistically significantly, from 2.05 mg/dm^3 to 192.65 mg/dm^3. The activity of cathepsin C, after the application of aminofluoride preparations, was decreased significantly in the case of Elmex green fluid (72.6%). The other preparations caused a smaller reduction in the activity of the enzyme, being 43.5% for Elmex gelee, 43.2% for Elmex red fluid and 39.7% for Fluormex rinse. The differences were statistically significant in all the cases, except for Fluormex rinse. Protein content was decreased only after the application of Elmex green fluid, but it was not a statistically significant difference.

Discussion

Biotoxicity of fluoride ions results mainly from their inhibitory effect on the activity of many enzymes, mostly oxidoreductases, transferases, hydrolases, Krebs cycle enzymes as well as those which lead to ATP production and synthesize protein and DNA [11-14]. This is associated with high chemical activity of F ion and its affinity to Ca^+ and Mg^+, which catalyze a number of enzymatic reactions. The environmental pH is of key importance for the activity of fluoride ions, which increases in the acid environment. In the present study, a slight increase in pH was found for each preparation. These results are convergent with the findings reported by other authors [15]. The studies on the relationship between periodontal diseases and the presence of proteolytic enzymes in the mouth may help elucidate the complex underlying mechanism of these diseases. These enzymes are involved in tissue degradation, by damaging collagen, elastin and fibronectin [16]. High activity of cathepsin B and L has been demonstrated in gingival tissue homogenates in periodontitis patients. Similar correlation has been found for cathepsin D [17-19].

The activity of salivary cathepsin C is most inhibited after the application of Elmex green fluid, even though the preparation contains a smaller amount of fluorides than the other preparations. The inhibitory effect of oral hygiene preparations on proteolytic enzymes in the saliva was found in *in vitro* studies, which revealed a decrease in the activity of human salivary cathepsin D in the presence of various concentrations of Blend-a-med toothpaste [20]. The inhibition of the activity of proteolytic enzymes after the application of various oral hygiene preparations may slow down the inflammatory processes in soft tissues.

Conclusions

1. Fluorides contained in oral hygiene preparations cause a reduction in the activity of cathepsin C and increase salivary pH.

2. Saliva can serve as a diagnostic material in the examination of the environmental exposure to fluorides.

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