Effect of chlorhexidine mouthrinse on cathepsin C activity in human saliva

Dąbrowska E[1]*, Letko M[2], Roszkowska-Jakimiec W[2], Letko R[1], Sadowski J[1]

[1] Department of Social and Preventive Dentistry, Medical University of Białystok, Poland
[2] Department of Instrumental Analysis, Medical University of Białystok, Poland

Abstract

Chlorhexidine is an active agent commonly used against dental plaque in the mouth apart from fluorides applied to prevent caries. It is contained in toothpastes and mouthrinses.

Purpose: The aim of the study was to assess the effect of mouthrinses containing chlorhexidine digluconate on the activity of cathepsin C in human saliva.

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 40 subjects (dentistry students; 30 women and 10 men), aged 19-24. Saliva was collected before the preparations were applied after rinsing the mouth with distilled water and following a single use of the preparations according to the producer's instructions, 8 samples for each preparation.

Results: The decrease of cathepsin C was observed for each preparation, but was the greatest after mouth rinsing with Kin Gingival (65.08%) and Corsodyl (58.00%).

Conclusions: The current study confirms this assumption by finding a decrease in cathepsin C activity after the use of chlorhexidine mouth rinses.

Key words: cathepsin C, chlorhexidine, human saliva, mouthrinses.

* CORRESPONDING AUTHOR:
Department of Social and Preventive Dentistry
Medical University of Białystok
ul. Akademicka 3, 15-276 Białystok, Poland
Tel. +48 085 748 57 95; fax: +48 085 748 57 96
e-mail: helpdentamb@tlen.pl

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Introduction

Chlorhexidine is an active agent commonly used against dental plaque in the mouth apart from fluorides applied to prevent caries [1-4].

Chlorhexidine is the longest and the most frequently used antibacterial and anti-inflammatory agent, being the focus of research as far back as the 50s of the previous century. It is considered to be one of the most effective antiseptics, decreasing dental plaque formation and inhabiting the development of gingivitis even when mechanical cleaning has been neglected. It is contained in toothpastes (Elgydium, Lacalut, KIN-Gingival) and mouth rinses (Corsodyl, Oral-B, Peridex, Parogencyl, Paroplak, Eludril, Oralsept, KIN-Gingival, Protefix, Gluxonit) [5].

Chlorhexidine, the cationic form of bis-biguanidine, occurs as gluconate or acetate. Charged positively, it shows high affinity for negative ions found in cell membranes of microorganisms. Chlorhexidine is more effective against the cell membranes of Gram-positive bacteria as they have a much higher charge than the Gram-negative ones. The hydrophobic part of chlorhexidine reacts with the structures of the bacterial cell membrane, disturbing its integrity and function. At a low concentration, molecules of the antiseptic bind to phosphate groups of lipopolysaccharides and to carboxy groups of proteins of the cell wall. This action interferes with cellular transport (potassium ions, amino acids and nucleotides) and disturbs metabolic processes [3]. Chlorhexidine indirectly affects the enzymatic function of dehydrogenase and ATP-ase present in the cell wall of bacteria [6].

At high concentrations of chlorhexidine, the cell membrane gets disrupted, which results in osmotic imbalance, escape of cytoplasmic components and cell death [7]. It can be thus assumed that high levels of this antiseptic exert a bactericidal effect, while low concentrations show a bacteriostatic action. Moreover, chlorhexidine binds to negatively charged mucous cells of the oral cavity, but as they differ in structure from the bacterial cells they remain intact. Bound to the mucous membrane or saliva proteins, chlorhexidine is gradually released in

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

	KIN-Gingival 0.12% chlorhexidine digluconate		**Corsodyl 0.1% chlorhexidine digluconate**		**Protefix 0.1% chlorhexidine digluconate**		**Eludril 0.1% chlorhexidine digluconate 0.5% chlorobutanole**		**Control distilled water**	
	Mean ±SD		Mean ±SD		Mean ±SD		Mean ±SD		Mean ±SD	
	Before	After	Before	After	Before	After	Before	After	Before	After
Cathepsin [nmol/ml]	0.1724 ±0.07	0.0602 ±0.02	0.0844 ±0.21	0.0346 ±0.03	0.0854 ±0.02	0.0542 ±0.01	0.0616 ±0.09	0.0496 ±0.06	0.1415 ±0.12	0.18875 ±0.08
Protein [mg/ml]	0.1506 ±0.06	0.125 ±0.03	0.2376 ±0.07	0.29 ±0.07	0.2068 ±0.08	0.1626 ±0.08	0.1378 ±0.05	0.1634 ±0.08	0.98725 ±0.19	0.9725 ±0.11

the presence of Ca+2. Thus, for a certain period of time, the oral cavity becomes a chlorhexidine reservoir, which prolongs its chemical activity in preparations [3,6,7].

In the inflammatory processes in the mouth, lysosomal proteolytic enzymes are released, inducing periodontal disorders. These enzymes include cathepsin C (dipeptidyl-peptidase I), an exopeptidase, which splits off dipeptides from the N-terminal of peptides [8]. Cathepsin C belongs to exogenic salivary peptidases, cleaves off p-nitroaniline (pNA) from dipeptide p-nitroanilides, has a cystein catalytic site and to be active needs chloride anions. It is also believed to have transferase properties [9]. Since no literature data are available on the effect of chlorhexidine gluconate on the activity of cathepsin C, we have decided to examine this relationship.

Objective

The aim of the study was to assess the effect of mouthrinses containing chlorhexidine digluconate on the activity of cathepsin C in human saliva.

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 40 subjects (dentistry students; 30 women and 10 men), aged 19-24. They were divided into 5 groups, each group receiving different preparation, and control group. The students were healthy, non-smoking, with no active foci of caries. In 4 groups of 8 students, chlorhexidine mouthrinses were used. In group 5 (control), distilled water was applied as a mouth rinse. Saliva was collected before the preparations were applied after rinsing the mouth with distilled water and following a single use of the preparations according to the producer's instructions, 8 samples for each preparation. Test-tubes with the saliva samples were frozen immediately and stored at a temp. of -18°C ÷ -24°C. After defrosting, the activity of cathepsin C (EC 3.4.14.1) was determined in non-fractionated saliva according to Plant with the use of Gly-Phe-pNA substrate. [10]. The enzyme activity was measured by assessing the amount of the obtained product and expressed in nmol/ml [11]; Bradford method was used to assess protein content [12].

The following preparations containing chlorhexidine digluconate were used: KIN-Gingival (0.12%), Corsodyl (1.1%), Protefix (0.1%), Eludril (1.1%). The study was approved by the local Bioethics Committee.

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 before and after application of the preparation, 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

The average values of the study parameters, cathepsin C activity and the amount of salivary protein calculated for all the study subjects have been presented in *Tab. 1*. Gender was not taken into account as the parameters did not differ significantly between women and men. Since all the preparations contained chlorhexidine digluconate in similar concentrations, they have been listed according to cathepsin C decrease caused by their action. The decrease was observed for each preparation, but was the greatest after mouth rinsing with KIN-Gingival (65.08%) and Corsodyl (58.00%). Protefix showed similar effects (36.53%). The slightest reduction in cathepsin C activity was found after the application of Eludril (19.48%), which may be explained by the fact that 0.5% chlorbutanol was added to this rinse. Most of the chlorhexidine preparations used caused a slight decrease in the amount of salivary protein. However, the results were not statistically significant.

Discussion

Chlorhexidine is one of the most effective antiseptics. It inhibits dental plaque formation and gingivitis even when mechanical cleaning has been neglected. It is contained in toothpastes and daily use mouthrinses. Chlorhexidine digluconate is the active component of the rinses. Our current findings demonstrate a reduction in cathepsin C activity after application of chlorhexidine-containing preparations. Previously, we demonstrated a distinct and statistically significant reduction in the enzyme activity due to fluoride preparations [13].

The decrease was larger for amino fluorides (72.6% for Elmex green liquid), probably due to higher bioactivity of amino

fluorides on cathepsin C, compared to chlorhexidine. The effect of fluoride preparations on salivary enzymes has been demonstrated in many studies. Kaczmarek [14-16], who studied the activity of such salivary enzymes as alpha-amylase, peroxidase and myeloperoxidase, obtained their reduction in the environment of fluoride ions suggesting high biochemical activity of the latter. A more substantial decrease in cathepsin C activity after amino fluorides demonstrated in our earlier study than after chlorhexidine seems to confirm a specific mechanism of fluorides involving inhibition of intra- and extra-cellular enzymes.

Literature data on cathepsin C and its effect on oral health are lacking and hence no discussion with the findings of other authors is possible. Research reports have revealed that the activity of cathepsins B, L, D intensifies in periodontal inflammatory states, suggesting involvement of these enzymes in tissue degradation, damage to collagen, elastin and fibronectin [17--20]. Only Etemadzadeh et al., who investigated chlorhexidine and aminofluoride-based preparations, found no changes in the activity of lysosomal enzymes, saliva pH or saliva secretion rate. However, they noted a smaller amount of dental plaque due to chlorhexidine application compared to aminofluorides [21]. The effect of chlorhexidine on bacterial plaque has been already investigated in clinical settings.

Dental plaque has the potential to bind substantial amounts of the antiseptic, whose proper concentrations may cause plaque disintegration and splitting from the enamel surface. Application of chlorhexidine varnish reduces colonisation of *Streptococcus* mutans in dental plaque [4]. Particularly sensitive even to low doses of chorhexidine are the bacteria *Streptoccocus* mutans, *Fusobacterium nucleatum*, *Streptoccocus sanguinis*, *Actinomyces*, *Lactobacillus*, *Enterococcus faecalis*, *Porphyromonas gingivialis*, *Prevotella intermedia*, as well as yeasts (*Candida albicans*), certain dermatophytes and *in vitro* viruses (Herpes simplex, HIV). Chlorhexidine gluconate in a mouthrinse caused a drop in aerobic and anaerobic bacterial cultures, with lower sensitivity of Gram(-) anaerobes [25].

A two-minute mouth rinsing with Skinsept was found to result in a considerable decrease in the number of the respective bacteria or even their elimination. Reduction in the bacterial population on the tongue surface and in saliva ranges between 81% and 99% [26,27].

During local irrigation with 0.3% antiseptic a decrease was observed in periodontal inflammatory symptoms, in dental plaque index, in gingival fluid index, sulcus bleeding index, probing depth and bacterial counts. Besides, chlorhexidine irrigations improve the epithelial attachment level due to the inhibitory effect of chlorhexidine on the enzymes involved in periodontitis: metalloproteinases, cathepsins, elastases [28,29].

Research on the relationship between periodontal diseases and the presence of proteolytic enzymes in the mouth may elucidate a complex mechanism of these diseases.

Conclusions

Considering our findings and the results reported by other authors it can be assumed that mouth rinses for everyday use can improve inflammatory states of periodontal tissues through inhibition of proteolytic enzymes. The current study confirms this assumption by finding a decrease in cathepsin C activity after the use of chlorhexidine mouth rinses.

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