

Assessment of salivary levels of the chosen exoglycosidases in patients with aggressive periodontitis after treatment with doxycycline

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Abstract

Purpose: The aim of the study was the clinical assessment of the periodontium in patients with aggressive periodontitis (AP) after treatment with doxycycline hyclate. Moreover, an attempt was made to evaluate the effect of the treatment on the salivary concentrations of β -glucuronidase, HEX, HEX A and HEX B in AP patients.

Material and methods: Sixteen patients with aggressive periodontitis, aged 28-45 years, were enrolled in the study. The patients were treated with a doxycycline hyclate preparation (Periostat) for 2 months at a dose of 20 mg twice a day. The clinical examination was performed twice, directly prior to pharmacological treatment and after its termination. The following clinical parameters were evaluated: the plaque index (PI), the sulcus bleeding index (SBI), the pocket probing depth (PPD) and the clinical attachment level (CAL). Biochemical determination of β -glucuronidase, HEX, HEX A and HEX B concentrations in non-stimulated saliva was performed before and after treatment.

Results: In AP patients, the values of PI, SBI and CAL before and after treatment were comparable. The mean pocket probing depth before treatment was 3.5 mm, which decreased significantly after treatment (3.2 mm). The values expressed as pKat/kg protein for specific enzymatic activities of HEX, HEX A, HEX B and β -glucuronidase in the saliva of AP patients before and after doxycycline treatment were similar.

Conclusions: A 2-month treatment with doxycycline is too short to obtain clinical changes. Although the assessment of the activity of such enzymes as β -glucuronidase,

HEX, HEX A and HEX B in the saliva of AP patients allows detection of periodontal inflammation, it cannot be used to determine the risk of its development and therefore has no practical significance.

Key words: aggressive periodontitis, doxycycline hyclate, proteolytic enzymes.

Introduction

Periodontal inflammations are progressive diseases of the tooth supporting structures [1]. Their pathogenesis is very complex, with dental plaque being the major etiologic factor. Over 500 species of bacteria have been identified in dental plaque but only several of them, especially *Actinobacillus actinomycetemcomitans* and the red complex bacteria cause periodontal tissue destruction [2]. Due to inflammatory reactions, numerous proteolytic enzymes which destroy matrix proteoglycans are released [3,4]. These enzymes belong to the class of exoglycosidases and include N-acetyl- β -hexosaminidase (HEX), β -galactosidase, α -mannosidase, α -fucosidase and sialidase, which split single monosaccharides off the non-reductive oligosaccharide terminal portion and are specific for one anomeric form of glycosyde bond. Together with endoglycosidases they form a series of reactions, in which the product of one reaction is the substrate of the subsequent one [5].

N-acetyl- β -hexosaminidase (HEX, NAG, E.C. 3.2.1.52) is the most active lysosomal enzyme. It hydrolyses saccharose chains of glycoconjugates, releases N-acetylglucosamines and N-acetylgalactosamines from various β -oligosaccharides of glycopeptides and glycoproteids, and during hyaluronic acid breakdown [6]. The presence of this enzyme has been found in the saliva, blood serum and plasma, the cerebrospinal fluid and articular fluid, as well as in many tissues and organs, e.g. in animal salivary glands [6-9]. It has been proved that HEX is produced in mucous and epithelial cells of outlet ducts in the sub-

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Table 1. Clinical parameters (mean, \pm standard deviation) in preliminary and follow-up examination

Parameter	Examination I	Examination II
PI	0.7 \pm 0.44	0.8 \pm 0.49*
SBI	43.1 \pm 17.39	46.3 \pm 18.48
PPD	3.5 \pm 0.77	3.1 \pm 0.67*
CAL	4.2 \pm 1.23	4.0 \pm 1.1

* – statistical difference between I and II examinations; PI – plaque index; SBI – sulcus bleeding index; PPD – periodontal pocket depth; CAL – clinical attachment level

mandibular salivary gland [8]. HEX is built of two polypeptide chains – α and β . A few N-acetyl- β -hexosaminidase isoenzymes have been isolated: A, B, C, I₁, I₂, P, S. HEX A contains α and β chains, HEX B and P have two β chains. Immunoenzymatic tests using anti-HEX B antibodies have allowed classification of isoenzymes B, I₁, I₂, P as one HEX B group [5].

β -glucuronidase (glucuronohydrolase of β -D-glucuronide E.C. 3.2.1.31) is responsible for the reaction that yields β -glucuronians – compounds of the glucuronic acid with phenol, alcohols and carboxy acids. Formation of such conjugates is a known method of detoxication. β -glucuronidase has been found in the secretion of parotid and submandibular glands [10]. Elevated levels of N-acetyl- β -hexosaminidase and β -glucuronidase have been observed in the gingival sulcus fluid, saliva and periodontal tissues of patients with periodontal disease [3,4,11-16].

In order to inhibit the disease and stabilize the attachment level, the periodontal treatment aims to decrease periodontal pocket pathogens [17]. It consists of three phases: preliminary, corrective and supportive. In the latter, the mechanical procedure reducing the number of bacteria is complemented with general pharmacotherapy, which can be either addressed against periodontal pocket pathogens or modulate the host response. The drug used to modulate the host response is doxycycline hyclate, which affects local inflammatory reactions through the release of enzymes, metalloproteinases (MMP) in the first place [18]. Administration of doxycycline to periodontitis patients caused flattening of the periodontal pocket depth (PPD), reduction in the clinical attachment (CAL) and decreased bleeding (SBI) [1,11,19-23].

Therefore, an attempt was made to clinically assess the periodontal status of patients with aggressive periodontitis (AP) after treatment with doxycycline hyclate. Moreover, we decided to evaluate the effect of the treatment on the salivary concentrations of β -glucuronidase, HEX, HEX A and HEX B in AP patients. Changes in the levels of these enzymes could be potentially used as inflammation reduction indices and serve as prognostic markers of the disease.

Material and methods

The study involved 16 patients with aggressive periodontitis, aged 28-45 years (10 women and 6 men). A few weeks before the start of the treatment all the patients underwent professional dental cleaning. Then, they were treated with a doxycycline

Table 2. Specific activity pKat/kg exoglycosidase protein (mean, \pm standard deviation) in preliminary and follow-up examination

Exoglycosidase	Examination I	Examination II
HEX	10.8 \pm 3.91	13.3 \pm 4.27
HEX A	6.8 \pm 2.62	6.9 \pm 2.95
HEX B	4.1 \pm 2.09	6.4 \pm 5.41
β -glucuronidase	4.2 \pm 2.27	5.0 \pm 1.52

hyclate-containing preparation (Periostat, CollaGenex, USA) for 2 months at a dose of 20 mg twice a day. The preliminary examination was performed directly before, while the check-up after the pharmacological treatment. A periodontal probe PCP 11 (Hu-Friedy, Finland) was used for examinations.

The following parameters were used for clinical assessment of the periodontium:

- the plaque index (PI) according to Silness and Løe [24],
- the sulcus bleeding index (SBI),
- the pocket probing depth (PPD) (in mm),
- the clinical attachment level (CAL) (in mm).

The biochemical methods used to determine the levels of β -glucuronidase, HEX, HEX A and HEX B in the non-stimulated saliva included:

- N-acetyl- β -hexosaminidase and its isoenzymes A and B – the method of Chatterjee et al. [25], as modified by Zwierz et al. [26], Department of Pharmaceutical Biochemistry, Medical University of Białystok,
- β -glucuronidase – the p-nitrophenolic method [27], in own modification, Department Pharmaceutical Biochemistry, Medical University of Białystok,
- Protein level was determined with Lowry method [28], Department of Pharmaceutical Biochemistry, Medical University of Białystok.

A packet SPSS 8.0 PL was used for statistical analysis of the results. The t-Student test for pairs was applied to compare changes in the parameters at time intervals in the respective groups. Differences were considered statistically significant for $p \leq 0.05$.

Results

In the study group of AP patients the PI and SBI values obtained before and after treatment with Periostat were comparable. The mean depth of periodontal pockets before treatment was 3.5 mm and decreased significantly after treatment to 3.2 mm ($p=0.0009$). The mean CAL value after treatment did not change. The numerical values for these clinical parameters have been listed in *Tab. 1*. The values expressed as pKat/kg protein for specific enzymatic activities of HEX, HEX A, HEX B and β -glucuronidase in the saliva of AP patients before and after doxycycline treatment were similar. The mean values of the enzymes and standard deviations have been presented in *Tab. 2* and *Fig. 1-4*.

Figure 1. Specific activity pKad/kg protein HEX before and after treatment

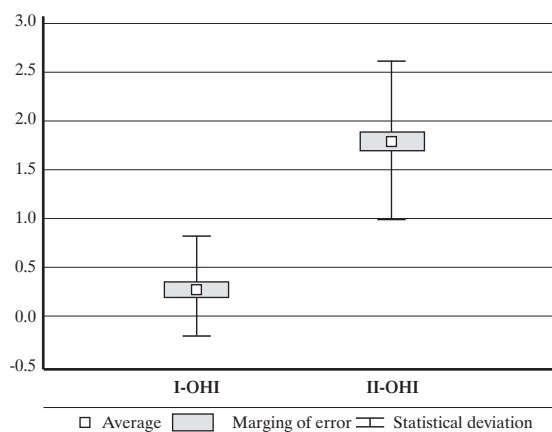


Figure 3. Specific activity pKad/kg protein HEX B before and after treatment

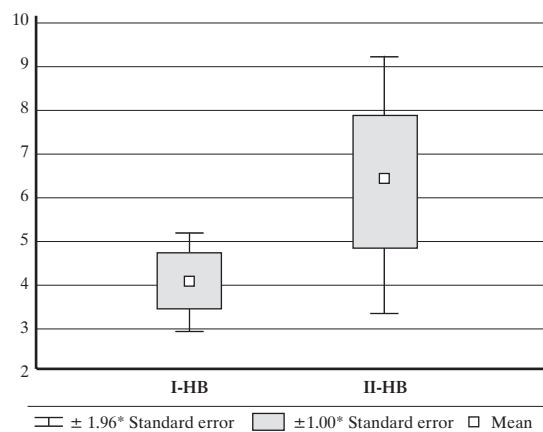


Figure 2. Specific activity pKad/kg protein HEX A before and after treatment

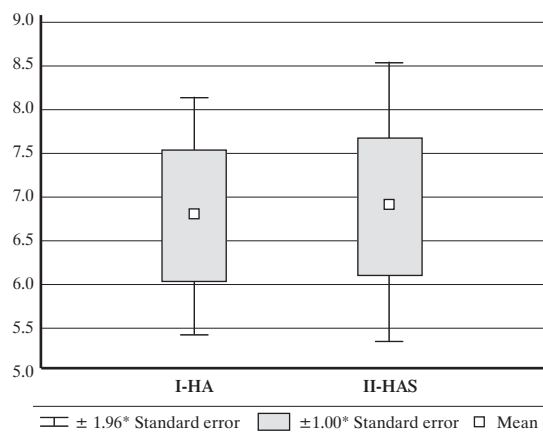
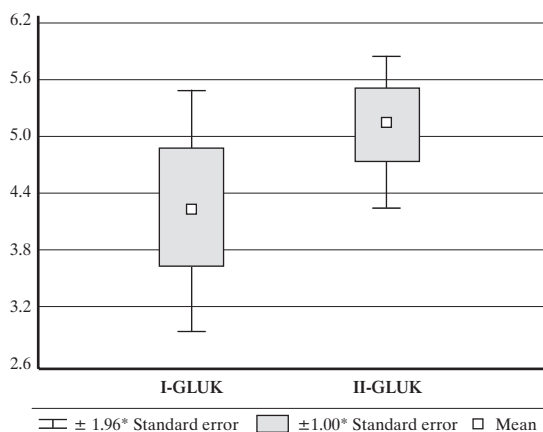


Figure 4. Specific activity pKad/kg protein β-glucuronidase before and after treatment



Discussion

Proper scaling, root planning and hygienic regime followed by patients are the standards of periodontal treatment. In 1998, periodontal therapy was complemented with doxycycline hyclate – a drug for use in combination with scaling and root planning [18,19,22]. It has been shown that a low 20 mg dose of doxycycline reduces the inflammatory process without undesired side-effects, i.e. it does not induce excessive growth of opportunistic flora, does not change bacterial sensitivity to antibiotics or induce resistance of bacteria in periodontal pockets [1,11,17,18,20-23]. The drug produces no side-effects, except for slight transitory gastric disorders, which are statistically insignificant as compared to the control group. This was also observed in our own material [21].

In the current study, no significant changes were found in the majority of the clinical parameters after doxycycline treatment. Statistically significantly reduced was only the pocket probing depth. Lack of differences in the clinical parameters can be explained by the fact that the patients were treated with the preparation for a short time, only for 2 months. Other authors have assessed the periodontal status after longer treatment with doxycycline hyclate. Ciancio et al. [21] showed considerable improvement in CAL, PPD and SBI after 12 months of

treatment, which according to the author may be caused by the inhibition of the release of the enzymes that damage collagen. The improvement in SBI is probably associated with better cohesion of collagen structure and not with anti-inflammatory effect of doxycycline. Long-term studies conducted by other authors have also demonstrated improvement in the clinical parameters [1,19,29]. However, reductions in PPD and CAL have been found to be greater in periodontal pockets that are at least 7 mm deep [11]. In our group of patients the mean PPD was 3.5 mm, with the highest PPD value being 4.7 mm, and hence the changes after treatment were insignificant.

The diagnosis of periodontal inflammations is based on the clinical and radiological examinations. However, as various inflammations show different activities, some attempts have been made to institute a number of differential diagnosis tests that would facilitate the disease prognosis by assessing the levels of various enzymes in the periodontal pocket. However, due to high price the test have not come into wide practical use. The assumption of the current study was the analysis of changes in the levels of the chosen enzymes: β-glucuronidase and N-acetyl-β-hexosaminidase in the saliva of AP patients after pharmacological treatment. These enzymes are present in the granules of primary neutrophils, whose migration to periodontal tissues and gingival sulcus is a particularly important consequence of dental

plaque accumulation [3,15]. The mean salivary enzyme levels did not change, although the analysis of the respective cases revealed an increase in the concentrations of β -glucuronidase and N-acetyl- β -hexosaminidase in 12 patients, while a decrease in 4. The increase in salivary enzymes in periodontitis patients can be caused by a number of factors and may occur despite the pharmacological treatment instituted. Lack of proper hygienic regime, which seems to be the most important, is associated with the accumulation of dental plaque and thus with PI increase. The increase in β -glucuronidase positively correlated with the presence of *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola*, i.e. the main components of the subgingival plaque [13,16].

Specificity and sensitivity are the major features of any diagnostic test. There are various levels of specificity and sensitivity for various β -glucuronidase values, e.g. the analysis show specificity of 32.2% and sensitivity of 91.3% for 40 units, and 84.9% and 42%, respectively, for 100 units. Therefore, some patients with poorly pronounced inflammatory changes can have high levels of β -glucuronidase, while those with advanced periodontitis – low values of this enzyme [4]. The study outcome can also be affected by contamination with blood, being an additional source of the enzymes [14]. According to some authors, the most intensive enzymatic growth is observed at the sites of the most severe inflammatory symptoms, where PPD is >5 mm [14,16]. Nieminen et al. [12] did not observe a statistically significant decrease in the concentration of three salivary exoglycosidases: β -HEX, β -galactosidase and α -glucosidase after 9 months of periodontal treatment [12]. Our current study was conducted on a group of patients previously subjected to periodontal treatment. It can be thus assumed that tests detecting β -glucuronidase can be useful in patients never before treated for periodontitis as well as in patients with gingivitis which may progress and develop into periodontitis [15]. Thus, the assessment of enzymatic activity allows only detection of inflammatory periodontal changes, which has no practical advantage.

Since the tests used to evaluate the salivary levels of exoglycosidases: β -glucuronidase, HEX, HEX A and HEX B vary in specificity and sensitivity and the results differ according to the disease advancement, they cannot be used as prognostic tests to determine the risk of the disease development.

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