Original research article/Artykuł oryginalny

N-acetyl-β-hexosaminidase in chronic tonsillitis and tonsillar hypertrophy

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A B S T R A C T

Background: The concentration and specific activity of N-acetyl-β-hexosaminidase (HEX) in palatine tonsils with chronic tonsillitis and tonsillar hypertrophy give insight in tonsillar tissue remodeling and constitute a potential marker for diagnosis and treatment of chronic tonsillitis and tonsillar hypertrophy. Aim: Determining the concentration and specific activity of N-acetyl-β-hexosaminidase in palatine tonsils with hypertrophy and chronic tonsillitis. Methods: HEX activity was analyzed by the method of Marciniak et al. with p-nitrophenyl N-acetyl-β-glucosaminepyranoside as a substrate. Results: The concentration and specific activity of HEX in palatine tonsils in patients with tonsillar hypertrophy and chronic tonsillitis both in childhood and adulthood significantly increase in comparison to healthy individuals. Conclusions: Our data demonstrate the presence of HEX in palatine tonsils and indicate on significant increase of its concentration and specific activity. Based on content and specific HEX activity we suggest that tonsils with hypertrophy and chronic tonsillitis should be treated as identical unit irrespectively of age.

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Introduction

The palatine tonsils are the largest component of Waldeyer tonsillar ring. The location of Waldeyer tonsillar ring and its design allow direct exposure of the immunologically active cells to foreign antigens entering the upper aerodigestive tract, which maximizes the immunologic response [1]. The tonsillar structures maximize the exposure of tissue to surface antigen, harbor debris and bacteria which may be the reason why tonsils are so commonly infected and hypertrophied. Chronic tonsillitis and tonsillar hypertrophy are often encountered diseases in otolaryngology, responsible for a significant proportion of childhood and adulthood illnesses. Although the criteria for tonsillectomy or subtotal (i.e. intracapsular) tonsillectomy have been proposed, otolaryngologist still debate the duration of conservative treatment and the necessity of performing the subtotal tonsillectomy [2, 3]. Therefore, there is a need for better understanding of palatine tonsils diseases. We suggest that one of such method may be the assessment of lysosomal exoglycosidases activity.

N-acetyl-β-hexosaminidase (HEX), the most active lysosomal exoglycosidase, contributes to degradation of a tonsillar oligosaccharide chains of glycoconjugates: glycoproteins, glycolipids and proteoglycans [4]. The increase of HEX activity was reported in patients with chronic inflammatory joint, kidney disease and salivary gland tumors [5–7]. HEX activity has been proposed as biochemical marker of tissue injury, inflammation, malignancy and alcohol abuse [8–14]. Therefore, we decided to investigate contribution of HEX activity in pathogenesis of chronic tonsillitis and tonsillar hypertrophy. The objectives of our study were as follows: evaluation of HEX presence in healthy palatine tonsils (C), tonsillar hypertrophy (H), chronic tonsillitis in children (TC) and adults (TA); comparison the HEX activity among C, H, TC, TA patients’ subsets.

Materials and methods

Palatine tonsils

Palatine tonsils were obtained from patients who underwent subtotal or total tonsillectomy due to tonsillar hypertrophy (H) (n = 15), chronic tonsillitis in children (TC) (n = 8) and chronic tonsillitis in adults (TA) (n = 22) in Department of Pediatric Otolaryngology, Medical University of Białystok, and Department of Otolaryngology Voivodship Hospital in Białystok. Healthy palatine tonsils, serving as a control (C) (n = 15), were obtained in Department of Forensic Medicine, Medical University of Białystok from young, healthy individuals, within 14 h after sudden decease. The study was approved by the local ethics committee, and all patients or parents of patients signed an informed consent form.

Tissue samples

The samples, removed from palatine tonsils, were washed with 0.9% NaCl, weighed and homogenized at 4 °C in an Ultra Turrax T8 homogenizer in 10 volumes of 0.15 M KCl containing 0.2% Triton X-100. The resulting homogenates were centrifuged at 10,000 × g for 30 min at 4 °C. The supernatants were used as crude enzyme solution.

Assay for exoglycosidase activity

The activity of HEX (EC 3.2.1.55) was determined by the method of Marciniak et al. [15]. The substrate for HEX was 6.67 mM solution of p-nitrophenyl-β-D-N-acetylgalcosamine-pyranoside (Sigma, St Louis, MO, USA), in 0.1 M citrate-phosphate buffer at pH 4.7, stored at –20 °C. The substrate was incubated for 60 min at 37 °C with appropriately diluted supernatant with 0.1 M citrate-phosphate buffer. The reaction was stopped by adding 0.2 M borate buffer, pH 9.8.

The measurements of the released p-nitrophenol were carried out at 405 nm using a spectrophotometer (Spekol 11, CARL ZEISS JENA). The concentrations of the HEX activity were expressed as nanokatals (nanomoles of p-nitrophenol released per second) per mL of tonsillar supernatant and specific activity as milikatals/kg of the supernatant proteins from tonsillar tissue.

Statistical analysis was performed using Statistica 9.0 (Statsoft, Cracov, Poland). Kruskal–Wallis ANOVA, Median and LSD tests were used to study the significant differences between groups. Statistical significance was defined as p ≤ 0.05.

Results

The concentration of the HEX activity in tissue with tonsillar hypertrophy (124.3 nkat/mL), chronic tonsillitis both in childhood (123.4 nkat/mL) and adulthood (128.7 nkat/mL) significantly increase in comparison to healthy palatine tonsils (78.1 nkat/mL). The concentration of the HEX activity is 1.5 times greater for both hypertrophied tonsils and chronic tonsillitis in children and 1.6 times greater for chronic tonsillitis in adults, in comparison to healthy tonsillar tissue (Table I, Fig. 1).

The specific HEX activity accounts for 18.6 mkat/kg of tonsillar supernatant proteins in the hypertrophied tonsils, 17.9 mkat/kg for chronic tonsillitis in children’s tonsillar tissue and 18.9 mkat/kg for chronic tonsillitis in adult’s tonsillar tissue. The specific HEX activity of tonsillar supernatant proteins is 1.6, 1.5 and 1.6 times greater, respectively in comparison to healthy tonsillar tissue (Table II, Fig. 2).

Discussion

The immune activity of the tonsillar tissues is most prominent from the ages of 4 to 10 and tends to involute after puberty. After involution, the secretory immune function of tonsillar tissues remains, but at decreased level. According to Paulussen et al. [16], due to important, but not completely investigated immune function of the tonsillar tissues and significant postoperative morbidity it is essential to limit indications for tonsillectomy. This raises the question: is there a difference in metabolism of tonsillar tissue in...
chronic tonsillitis and tonsillar hypertrophy. In the presented study, we have attempted to answer above question by determination of N-acetyl-β-hexosaminidase (HEX) that may contribute to degradation of the tonsillar connective tissue components, inter alia glycoconjugates, in chronic tonsillitis and tonsillar hypertrophy.

Our study demonstrated the presence of highly active HEX in healthy and pathologic tonsillar tissue both in children and adults, which suggest that indeed, HEX in tonsillar tissue is involved in degradation of oligosaccharide chains of glycoconjugates. The latter include glycoproteins and glycolipids [17] constituting cellular membranes, as well as heteropolysaccharide chains of glycosaminoglycans and proteoglycans constituting with glycoproteins extracellular matrix. Oligosaccharide chains are released from glycoconjugates by endoglycosidases (e.g. hyaluronidase or chondroitinases in the case of glycosaminoglycans) and then successively cleaved at the non-reducing end of oligosaccharide chains by concerted action of exoglycosidases, and in the case of HEX, resulting in release single N-acetylglucosamine or N-acetylgalactosamine units [18].

Our results indicate that concentration and HEX specific activity in hypertrophied tonsils and chronic tonsillitis in both children and adults were significantly greater than in healthy individuals, whereas there was only negligible difference in HEX concentration and specific activity between both hypertrophied tonsils and individuals with chronic tonsillitis. Our results accord to those obtained by Zochzwierz and Rudobielska [6] regarding chronic renal insufficiency and Popko et al. [5] concerning rheumatoid arthritis and juvenile idiopathic arthritis.

On the basis of HEX activity, it can be concluded that tonsillar hypertrophy and chronic tonsillitis can be treated as identical units. We assume therefore that although the subtotal tonsillectomy may offer a decreased rate of postoperative morbidity [19], in the long-standing outcome it can contribute to transformation of preserved tonsillar tissue into chronic tonsillitis.

Furthermore, we have observed that there is no significant difference in concentration and HEX specific activity between chronic tonsillitis in children and adults. We hypothesize therefore that the tonsillar remodeling is

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| Fig. 1 – The HEX activity (nkat/mL) in human palatine tonsils |

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<th>Table II – The specific HEX activity (mkat/kg protein) in human palatine tonsils</th>
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equally intensive both in childhood and in adulthood. Thus, there is an increasing body of evidence disapproving the need of subtotal tonsillectomy.

We have provided evidence that not only chronic tonsillitis but also tonsillar hypertrophy comparably accelerate the tonsillar tissue remodeling, reflected in increased HEX activity, eventually leading to tonsillar tissue impairment. Theoretically, the removal of pathological tonsils should decrease the HEX activity either in blood serum, saliva or urine. Therefore, determination the HEX concentration and specific activity in body fluid before and after tonsillectomy could constitute a plausible alternative to HEX examination in palatine tonsils in vivo. Further study on this hypothesis is needed, but it may offer additional details about specific features, monitoring and treatment of particular tonsillar diseases. Our assumption agrees with a study of Garcia Callejo et al. [20] who examined activity of superoxide dismutase (SOD) in blood and palatine tonsils before and after tonsillectomy. They concluded that SOD activity in palatine tonsils and/or peripheral blood increases proportionally to infections incidence, which allows detecting patients with functional tonsillar tissue damage, and objectively recommending tonsillectomy or monitoring clinical response for a therapy.

As the determination the HEX activity is a simple, robust, non-invasive and inexpensive, we speculate that the HEX activity in body fluids determined before and after tonsillectomy could be a potential marker for monitoring the course of particular palatine tonsils’ diseases.

Conclusions

In the present study, we have demonstrated the presence of HEX in palatine tonsils and depicted its concentration and specific activity differences between healthy individuals and those with chronic tonsillitis and tonsillar hypertrophy. We concluded that the significant increase of HEX concentration and specific activity in well-defined group subsets prove increased glycoconjugates’ catabolism contributing to tonsillar tissue damage. Our results established a sound basis for treating tonsillar hypertrophy and chronic tonsillitis as identical unit irrespectively of age. Furthermore, we suggest that it would be of a particular interest to examine HEX concentration and specific activity in body fluids before and after tonsillectomy. It could constitute a potential marker for diagnosis and treatment of chronic tonsillitis and tonsillar hypertrophy.

Authors' contributions/Wkład autorów

MZ was responsible for study design, data collection and interpretation, statistical analysis, acceptance of final manuscript version, literature search, funds collection. AM was responsible for study design, data collection and interpretation, acceptance of final manuscript version, literature search. MK was responsible for study design, data collection, acceptance of final manuscript version, literature search. AN-J was responsible for data collection, statistical analysis, acceptance of final manuscript version, literature search. JM, MB and AZ were responsible for data collection, statistical analysis, literature search. LM was responsible for statistical analysis, data interpretation literature search. AJ and TJ were responsible for statistical analysis literature search. KZ and SS were responsible for data interpretation, acceptance of final manuscript version, funds collection.

Financial support/Finansowanie

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Conflict of interest/Konflikt interesu

None declared.

Ethics/Etyka

The work described in this article have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

REFERENCES/PIŚMIENNICTWO


