Exoglycosidase markers of diseases

Sylwia Chołnowska*, Alina Kępka†, Sławomir Dariusz Szajda‡, Napoleon Waszkiewicz§, Marcin Bierc∥ and Krzysztof Zwierz∗†

*Medical Institute, College of Computer Science and Business Administration, 18-400 Łomża, Poland, †Department of Biochemistry and Experimental Medicine, the Children’s Memorial Health Institute, 04-730, Warsaw, Poland, ‡Department of Pharmaceutical Biochemistry, Medical University, 15-230 Białystok, Poland, §Department of Psychiatry, Medical University, 16-070 Białystok, Poland, and ∥Department of Stomatological Surgery, Medical University, 15-276 Białystok, Poland

Abstract

Exoglycosidases are hydrolases involved in lysosomal degradation of oligosaccharide chains of glycoconjugates (glycoproteins, glycolipids and proteoglycans). In tissues and body fluids, a higher exoglycosidase specific activity is found in N-acetyl-β-hexosaminidase, than β-galactosidase, α-L-fucosidase, β-galactosidase, α-mannosidase and α-glucosidase. Determination of exoglycosidases (especially N-acetyl-β-hexosaminidase and β-glucuronidase) in body fluids could be an inexpensive, easy to perform and sensitive test for pathological evaluation, as well as in screening and monitoring many diseases, including alcohol abuse, risk of arteriosclerosis, bacterial infections (e.g. Lyme borreliosis), chronic inflammatory processes, such as rheumatoid arthritis and juvenile idiopathic arthritis, asthma, autoimmune hepatitis and primary biliary cirrhosis, as well as cancers.

Introduction

Exoglycosidases are hydrolases involved in post-translational modifications of glycoproteins and degradation of glycoconjugates (glycoproteins, glycolipids and proteoglycans). Post-translational modification of glycoprotein sugar chains occurs in the endoplasmic reticulum and Golgi apparatus [1]. Degradation of glycoconjugates takes place in several organelles, including proteosomes, where short-lived and incorrectly folded glycoproteins derived from the nucleus, cytoplasm and endoplasmic reticulum are degraded [2]. However, the lysosomes are the main site for degradation of the oligosaccharide chains of glycoconjugates [3].

In lysosomes there is a gradual release of terminal sugars from glycoconjugate oligosaccharide chains [4]. Since glucuronic acids are mainly incorporated into proteoglycans and mannoside mainly to asparagine-linked glycoproteins, the activity of GluA (β-glucuronidase) is a specific marker for the degradation of proteoglycans and MAN (α-mannosidase) for the degradation of asparagine-linked glycoproteins. As all glycoconjugates have N-acetylhexosamines, HEX (N-acetyl-β-hexosaminidase) is a universal marker for degradation of glycoconjugates. In tissues, e.g. human gastric mucosa, GLU (α-glucosidase) exhibits the lowest specific activity. Activities of MAN exceeded GLU 1.9 times, GAL (β-galactosidase) 5.5 times, FUC (α-L-fucosidase) 11 times and HEX 120 times [5]. In cultured human synovial fibroblasts from injured knee joints, the specific activity of exoglycosidases increases in a defined order: FUC, GAL, GluA and HEX [6]. The intensity of lysosomal degradation of glycoconjugate oligosaccharide chains depends on the quality of the lysosomal exoglycosidases and regulatory substances [6,7].

Tissue exoglycosidase activity as a marker of many diseases

The activity of various exoglycosidases in surgical specimens reflects damage to the tissue caused by different pathological processes. An example is HEX, a novel potential marker of cholesteatoma [8]. The mean activity of HEX in cholesteatoma (68.55 ± 30.77 nkat/g of wet tissue) was significantly higher than in skin (31.79 ± 10.02 nkat/g of wet tissue). [9] A correlation between HEX activity in cholesteatoma and bone resorption was also demonstrated [9].

An evaluation of the activity of lysosomal exoglycosidases in tissue may be helpful in establishing pathogenesis and treatment of some diseases. An example is the pathogenesis of nasal polyps [10]. Nasal polyps are smooth outgrowths assuming the shape of grapes, formed from the altered nasal mucosa, which project into the lumen of the nasal cavity, limiting air flow. To assess the utility of lysosomal exoglycosidases in the elucidation of nasal polyp

Key words: alcoholism, cancer, exoglycosidase marker, inflammation.

Abbreviations used: EPH gestosis, oedema-proteinuria-hypertension gestosis; FUC, α-L-fucosidase; GAL, β-galactosidase; GLU, α-glucosidase; GluA, β-glucuronidase; HEX, N-acetyl-β-hexosaminidase; MAN, α-mannosidase.

To whom correspondence should be addressed (email kzwie@umwb.edu.pl).

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pathogenesis, we determined the concentration of activity and specific activity of exoglycosidases in nasal polyps. We found a significant decrease in the concentration of activity (in comparison with control) for several exoglycosidases and simultaneously increased specific activity of HEX A. The decrease in concentration of the activities of exoglycosidases in polyp tissue, without significant changes in specific activities, contradicts the theory of fully symptomatic inflammation in the nasal polyps and may confirm the neoplastic theory of the nasal polyps pathogenesis [10].

The activity of exoglycosidases may be helpful in understanding and treating diseases of palatine tonsils. The palatine tonsils participate in the composition of the Waldeyer tonsillar ring, which maximizes immunological responses [11] by exposure of the nasopharyngeal lymphatic tissue to surface antigens by harbouring debris and bacteria; the reason why tonsils are so frequently infected and hypertrophied. Diseased palatine tonsils are responsible for a significant proportion of childhood and adult diseases and, consequently, require either non-invasive or surgical treatment. Although criteria for tonsillectomy or subtotal (i.e. intracapsular) tonsillectomy have been proposed, otolaryngologists still debate the duration of non-invasive treatment and the necessity of performing a subtotal tonsillectomy [12]. Popko [13] has attempted to answer the above question by determining the activity of exoglycosidases in chronic tonsillitis and hypertrophy of tonsils. Popko [13] reported an almost identical increase (in comparison with control) in the mean concentration of exoglycosidases activity: 1.65 times in chronic childhood tonsillitis, 1.64 times in chronic adult tonsillitis and 1.63 times in lymphatic hypertrophy. The mean specific activities (in comparison with control) of exoglycosidase activity increased 1.70 times in chronic adult tonsillitis, 1.66 times in hypertrophy of tonsils and 1.64 times in chronic children tonsillitis. On the basis of exoglycosidase activity, it can be concluded that tonsillar hypertrophy, chronic childhood and adult tonsillitis can be treated as identical conditions.

Differentiation between benign and malignant growth is crucial in the prognosis and strategy of treatment of neoplastic diseases. The activity of exoglycosidases in tissues obtained during surgery may be a helpful marker in distinguishing between benign and slowly growing tumours, and those which are intensely growing intracranial tumours. Wielgat et al. [14] reported that the activities of exoglycosidases were significantly increased in malignant glial tumours, in comparison with normal brain tissue and non-glial tumours. The highest activities of exoglycosidases were observed in high-grade gliomas and a positive correlation between HEX A, as well as HEX B, activities, and the degree of malignancy of primary gliomas and meningiomas were observed. These authors suggested that activity of exoglycosidases in brain tumours is dependent on the phase of their development [14].

A significant increase in HEX, HEX A and HEX B activities in human pleomorphic adenoma (in comparison with healthy salivary glands) was reported by Borzym-Kluczyk et al. [15]. After separation by isoelectrofocusing, they found some changes in the pattern of HEX B isoforms, but there were no significant differences in the amount of HEX A and HEX B isoforms between pleomorphic adenoma and healthy tissue.

In contrast with the majority of studies on the activities of lysosomal exoglycosidases in neoplastic tissues, which have reported increases in exoglycosidase activity [14,15], Borzym-Kluczyk et al. [16] observed a significant decrease in HEX activity in renal cancer and transitional tissue, in comparison with normal renal tissue. The possible explanation for this observation is that renal cancer cells derive from proximal convoluted tubule, and the increased breakdown of the tubular cells caused by cancerous process may release HEX and its isoenzymes into the urine.

The activity of HEX in placentas may be a marker of abnormalities threatening pregnancy. In human placentas collected after delivery from women in puerperium with symptoms of prolonged pregnancy or complicated by EPH (oedema-proteinuria-hypertension) gestosis, the activity of HEX was significantly higher than in placentas from normal pregnancy. HEX A dominated in placentas from normal and prolonged pregnancy, but HEX B dominated in placentas with EPH gestosis [17]. The activity of HEX in the serum of pregnant women may be a marker of imminent abortion, where it is significantly higher than in normal pregnancy. It may also be a marker of missed abortion, where a significant decrease in serum HEX activity (in comparison with normal pregnancy) was observed [18].

**Body fluid exoglycosidases as disease markers**

A proportion of lysosomal hydrolases are delivered from lysosomes to the cell surface. Some of these are not returned to lysosomes and are instead secreted into the extracellular fluid [1]. The pattern of secreted exoglycosidases in synovial cells is similar to their activity in tissue [6]. Similar to tissues, in body fluids (serum, urine, synovial fluid and saliva), the highest activity is found for HEX and GluA [6,19]. The activities of lysosomal exoglycosidases in body fluids may be markers of both storage [1,7] and degradative [1] processes.

Chronic intoxication of rats by Cd (cadmium), at doses related to human chronic environmental and occupational exposures, induces dose-related kidney proximal tubular damage, confirmed biochemically and histopathologically. In rats exposed to 5 mg of Cd/litre of drinking water (related to environmental exposure), a significant increase in urinary HEX and HEX B occurred after 12 weeks of the experiment. However, in the urine of rats exposed to 50 mg of Cd/litre of drinking water (related to occupational exposure), a significant increase in urinary activity of HEX and HEX B occurred after only 6 weeks of exposure [20]. In a rat model of chronic Cd intoxication, we found a strong correlation between urinary HEX and HEX B activity, as well as between HEX and HEX B activity, and Cd concentration
in urine. Our results suggest the utility of HEX and HEX B activity in urine as a sensitive, simple and inexpensive marker for monitoring the chronic exposure of animals to Cd [21]. Others use HEX for monitoring chronic Cd exposure of factory workers, e.g. where particular pigments are used or Cd batteries are produced [22].

The increase in serum and urinary activity of HEX is a sensitive and inexpensive marker of alcohol abuse [23,24]. HEX B in serum and total HEX in urine are very sensitive markers of chronic heavy drinking and problem drinking [25], as well as single bingeing sessions [26]. HEX B in serum has 90% specificity and 70–90% sensitivity in the detection of alcohol abuse [25]. After chronic alcohol intoxication, the activity of HEX B in serum normalizes after 8 days and in urine after 1 month [25]. Activities of exoglycosidases in serum and urine are very significant markers of alcohol abuse, as they give objective information about the range and changes in alcohol consumption over time. They may also be very useful in the diagnosis of unconscious and seriously injured patients, as well as during post-mortem examinations. Exoglycosidase markers may be helpful in the differential diagnosis of psychiatric disorders and monitoring the efficiency of therapy during alcohol abuse. Exoglycosidase markers may be used to provide an early diagnosis of drinking and the return to drinking. Exoglycosidase markers may be helpful in setting the limits of safe drinking, and are additionally a powerful argument and motivation for changing the style of drinking to one less injurious.

A significant increase in the serum activity of HEX A in smokers may be a marker of the risk of arteriosclerosis [27]. An increase in the serum activity of exoglycosidases may be a marker of bacterial infections (e.g. Lyme borreliosis) [19] or chronic inflammatory processes such as rheumatoid arthritis and juvenile idiopathic arthritis [28] or asthma [29]. Increased activity of HEX in serum may be a marker of autoimmune hepatitis and primary biliary cirrhosis [30], as its activity correlates with biochemical and morphological parameters in autoimmune liver diseases [31].

An increased activity of serum and urinary exoglycosidases (especially HEX) may be a cancer marker [32]. Szajda et al. [33] have reported a significant increase in the concentration the activity of HEX and FUC (P < 0.001), as well as GAL (P < 0.022), in the serum of patients with colon cancer, in comparison with their activities in the serum of healthy controls. The ROC (receiver operator characteristic curve) analysis of the results determining HEX, FUC and GAL activity in the serum of patients with colon cancer had a high diagnostic value. Szajda et al. [33] calculated that the activity of HEX in the serum of colon cancer patients had 100% sensitivity and specificity, FUC had 85% sensitivity and 76% specificity, and GAL had 60% sensitivity and 76% specificity. In the urine of colon cancer patients, they found significantly increased HEX (P < 0.0003) and GAL (P < 0.002) activities. Activities of HEX and GAL in the urine of colon cancer patients have a high diagnostic value. The HEX concentration in urine had 85% sensitivity and 86% sensitivity, and the GAL concentration had 86% sensitivity and 93% specificity. Lysosomal exoglycosidases in serum and urine may be useful in diagnosing not only colon cancer, but also pancreatic cancer [34]. Significant increases in the activity of HEX A, GAL and FUC were reported in the saliva of patients with HIV infection, as well as a significant decrease in the activity of HEX B [35]. The increase in salivary activity of lysosomal exoglycosidases can be used as a marker of increased catabolism of oral cavity glycoconjugates caused by HIV and accompanying bacterial infections resulting from poor oral hygiene [35]. The specific activity of GluA in the saliva of smoking and non-smoking patients with Type 1 diabetes was significantly higher than GluA activity in the saliva of smoking controls without Type 1 diabetes and has a tendency to increase in relation to their activity in non-smoking healthy subjects. In a similar fashion to other exoglycosidases, it may be postulated that an increase in the activity of salivary exoglycosidases may be a useful marker for the qualification of these patients to a periodontitis risk group [36]. It is noteworthy that activities of salivary GAL and FUC in Type 2 diabetes decreases significantly and HEX, as well as MAN, have a tendency to decrease with age. This should be taken into consideration during the evaluation of salivary exoglycosidase activity as a marker of periodontal diseases [37].

HEX is valuable marker in veterinary and experimental medicine. HEX activity in camel’s milk was more effective in predicting the bacteriological status of the quarter than serum albumin [38]. Activity of HEX in cow’s milk may be a marker of subclinical mastitis caused by streptococci and the efficiency of antibiotic treatment [39]. The activity of HEX in cow’s milk is recommended as a marker of acute mastitis and as an inflammatory parameter during pharmacodynamic studies in addition to somatic cell count [40]. However, somatic cell count gave a better indication of the presence of pathogenic micro-organisms in camel’s milk than did HEX [41]. HEX provides versatile marker for secretion in studies of tear production in vivo and in vitro utilizing the rabbit as a model organism [42].

Concluding remarks

Increases in the activity of exoglycosidases (mostly HEX) in body fluids may be inexpensive and sensitive markers for preliminary screening of many diseases, including detection and monitoring of alcohol abuse, infections and cancers. The activities of exoglycosidases in body fluids could be especially valuable in monitoring chronic diseases, as urine, faeces, saliva and milk can be obtained by non-invasive methods.

Acknowledgement

We are grateful to Dr Catherine Merry from Manchester University for a critical reading of the paper before submission.
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Received 7 September 2010
doi: 10.1042/BST0390-046