Activity of lysosomal exoglycosidases in saliva of patients with HIV infection

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Abstract

Purpose: The aim of this work was to evaluate the influence of HIV infection on the catabolism of glycoconjugates in the oral cavity, by determination of the activity of lysosomal exoglycosidases in mixed saliva.

Method: The specific activities of the following exoglycosidases were tested: N-acetyl-β-hexosaminidase (HEX), its isoenzymes A (HEX-A) and B (HEX-B), α-mannosidase (MAN), β-galactosidase (GAL) and α-fucosidase (FUC).

Result: A significant increase of activity of HEX-A, GAL and FUC, and a significant decrease of the activity of HEX-B was found, but no significant changes in the HEX and MAN activity we noted.

Conclusion: Our results indicate that following HIV infection, there is probably an increased rate of catabolism of glycoconjugates in saliva resulting from changes in the proportions of the activity of isoenzymes A and B of N-acetyl-β-hexosaminidase, β-galactosidase and α-fucosidase. An increase of HEX-A activity can implicate the beginning of neoplastic changes developing in the oral cavity.

Key words: HIV, human saliva, lysosomal exoglycosidases.

Introduction

Glycoproteins and glycolipids form an integral part of the membranes of cells lining the oral cavity, and together with proteoglycans are present in teeth and the intercellular matrix of gingival's connective tissue [1]. Glycoproteins are also components of viral envelopes e.g. HIV [2]. Biosynthesis of host and viral glycoproteins take place in the endoplasmatic reticulum and Golgi apparatus [3], by concerted action of sugar transferases and glycosidases [4]. Degradation the oligosaccharide chains of glycoconjugates is performed by aminohydrolases, endoglycosidases and lysosomal exoglycosidases [5,6]. Inside lysosome glycoproteins are broken down by a combined action of proteases and exoglycosidases: neuraminidase (sialidase), N-acetyl-β-hexosaminidase (HEX), β-glucuronidase, β-galactosidase, α-fucosidase and α-mannosidase, which release neuraminic (sialic) acid, N-acetyl-hexosamines (N-acetylglucosamine and N-acetylgalactosamine), glucuronic acid, galactose, fucose and mannose, from non-reducing ends of oligosaccharide chains, respectively [7]. N-acetyl-β-D-hexosaminidase (HEX) and its isoenzymes A (HEX-A) and B (HEX-B) are most active of lysosomal exoglycosidases [8]. Isoenzyme A of N-acetyl-β-D-hexosaminidase is thermolabile and N-acetyl-β-D-hexosaminidase B is thermostabile form of HEX. As HIV infection is followed by neoplastic changes [9], we were interested in early diagnosis of transition to neoplasms, by exploiting significant differences in activity of HEX isoenzymes between normal and neoplastic tissues. An increase in HEX-A activity in comparison to HEX-B was observed by Borzym-Kluczyk et al. [10] in the renal tissue, serum and urine patients with renal cancer, Eden et al. [11] in acute undifferentiated leukaemia and Gil-Martin et al. [12] in human gastric adenocarcinoma. In inflammatory processes changes in proportion between HEX-A and HEX-B are not significant [13-15]. In healthy people, the activity of lysosomal exoglycosidases in periodontal tissues is low, but sufficient to maintain a steady state of glycoconjugates metabolism [6].

It has been estimated that worldwide about 14,000 people are infected each day by type 1 human immunodeficiency virus (HIV-1). The WHO estimated that 39.4 mln people all over the world were suffering from AIDS at the end of 2004

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[16]. However, therapy is limited by the number of drugs currently available. The drugs present on the market act in different ways; some pharmaceuticals act against the HIV virus, by blocking entrance to the host cells. One target which is involved in blocking viral entry into host cells are glucosidases. They has been recently explored because the biosynthesis of oligosaccharide chains of viral envelope glycoproteins depends on the activity of glucosidases and sugar transferases, which are also involved in the biosynthesis of glycoproteins responsible for the transport of viral particles from the cell and in adherence to and infection of new cells [17]. Hart et al. studied the effects of inhibitors of endoplasmatic reticulum glucosidases and Golgi mannosidase as well as neuraminidase on the interaction between HIV and mannose-binding lectin, a C-type lectin component of the human innate immune system, which binds to the gp120 envelope glycoprotein of HIV-1 [18]. The activities of β-hexosaminidase (using a 4-methylumbelliferyl-β-N--acetylglucopyranoside substrate) and of α-mannosidase and β-mannosidaseand were studied by Costanzi et al. who found that the activities of these enzymes were significantly higher in the serum of patients at the C3 stage of disease than in controls. No significant differences were observed in the activity of beta-glucuronidase or β-galactosidase [19].

HIV infection can coexist with periodontitis and neoplasms in the oral cavity [9,20,21].

Bacteria of dental plaque, the so-called bacteria of "red complex" (*Porphyromonas gingivalis*, *Taneralla forsythensis*, *Treponema denticola*) participating in the pathology of periodontitis. The chronic periodontitis is accompanied by an accumulation of neutrophils, macrophages and lymphocytes which take part in destruction of periodontal tissues. The increase in activity of proteolytic enzymes and enzymes degrading glycoconjugates which intensifies the destruction of connective tissue and bone in alveolar processes may cause increase in activity of lysosomal enzymes in serum and saliva [13].

Saliva is used for immunological and biochemical diagnosis and prognosis of oral changes in type I diabetes [22], diseases of the periodontium [23,24], and in Sjogren's syndrome, as it is easily accessible and a collection is a non-invasive procedure. Therefore, the aim of the present work is to evaluate the influence of the HIV infection on the catabolism of glycoconjugates in oral cavity by determination of the activity of lysosomal exoglycosidases in saliva from HIV patients.

Material and methods

The samples were 3-5 ml of mixed saliva collected from 68 patients by a spitting method without mechanical and chemical stimulation, not earlier than 1 hour and not later than 3 hours after a meal.

Control group (C) – 34 healthy patients, aged 21-48, extracted teeth – 3.35, need for extraction – 0.35, need for restorative and surgical treatment – 4.98. Any drugs were taken at least 8 hours before investigation.

Study group (HIV) – 34 HIV infected patients, aged 20-53, extracted teeth – 9.59 (CD 4 >500 – 6.25/8 patients; 200-500 – 9.19/27 patients; <200 – 12.64/14 patients), need for extraction – 2.10 (CD 4 >500 – 3.00/8 patients; 200-500 – 1.93/27 patients; <200 – 2.07/14 patients), need for restorative and surgical treatment – 11.18 (CD 4 >500 – 13.00/8 patients; 200-500 – 11.07/27 patients; <200 – 10.43/14 patients).

Table 1. **The activity of HEX, HEX-A, HEX-B, GAL, FUCMAN (μKat/kg protein) and concentration of protein (mg/ml) in saliva of the HIV patients**

The activity of enzymes μKat/kg protein (±SD)			
Control HIV			
HEX	mean	19.286±3.24	19.302±4.08
	p	~	
HEX A	mean	7.525±2.86	15.102±4.06
	p	0.047	
HEX B	mean	11.968±3.74	7.368±2.01
	p	0.000182	
GAL	mean	1.589±0.28	20.154±5.87
	p	0.00002	
FUC	mean	1.7131±0.75	1.925±0.57
	p	0.00115	
MAN	mean	2.423±0.97	2.384±1.24
	p	~	
Concentration of protein (mg/ml)			
protein	mean	1.9764±0.13	1.7614±0.19
	p	0.000683	

The collected saliva was centrifuged at 12,000 x g for 30 minutes at 4ºC. The resulting supernatant was divided into 100 μl portions and stored at -80ºC.

Determination of the activity (μKat/kg of protein) of N-acetyl-β-hexosaminidase (HEX), thermolabile isoenzyme A (HEX-A), thermostabile isoenzyme B (HEX-B), α-mannosidase (MAN), β-galactosidase (GAL) and α-fucosidase (FUC) in supernatants, was performed according to Zwierz et al. method [25]. The protein content (mg/ml) was determined by the Lowry method with bovine serum albumin as a standard [26]. All determinations were performed in duplicate.

The results were analyzed by a Statistica 6.0 StatSoft (Cracow, Poland) according to ANOVA and a post-hoc test (test NIR). The statistical significance of differences was regarded to be $p<0.05$.

This study was performed with the content of the Bioethical Commission of the Medical University of Białystok.

Results

Tab. 1 shows that no changes in the activity of HEX in saliva of patients with HIV infection (in comparison to activity of HEX in saliva of control group) was noted. However, the activity of thermolabile HEX-A in the HIV patients' saliva is over two times higher than the activity of HEX-A in the saliva of control group while the thermostabile HEX-B is 1.6 times lower in saliva of the HIV infected patients than in the saliva of control group. The activity of α-galactosidase and the α-fucosidase in the saliva of HIV infected patients is statistically higher than the activity of these enzymes in the saliva of the control group. The activity of α-mannosidase in the saliva of HIV infected patients does not differ significantly from the activity

***Table 2*. Activity of exoglycosidases calculated per volume of received saliva (μKat/kg protein/ml)**

The activity of enzymes μKat/kg protein/ml			
		Control	HIV
HEX	mean	6.248	5.939
HEX A	mean	1.835	3.107
HEX B	mean	2.3936	1.479
GAL	mean	0.496	5.447
FUC	mean	0.361	0.458
MAN	mean	0.502	0.599

of α-mannosidase in saliva of the control group. The mean concentration of the proteins in the saliva of HIV infected patients presents significant decrease in comparison to concentration of proteins in saliva of control group. *Tab. 2* shows activity of exoglycosidases calculated per volume of received saliva.

Discussion

It is established that HIV infects cells possessing the receptor CD 4, and in the case of lymphocytes deficient in CD 4, also through other receptors, such as the mannose or galactose receptors. The major determinant of viral tropism is at the entry level. This occurs only if the appropriate coreceptor is present. Entry of HIV-1 into its CD4+ target cells requires fusion/entry cofactors. Recently, the seven-transmembrane, G protein-coupled chemokine receptors CXCR4 and CCRS have been identified as cofactors for fusion and entry of T cell (T)3-tropic and macrophage (M)-tropic strains of HIV-1, respectively, into CD4+ cells [27-32].

CCR5 is the major coreceptor for HIV transmission in vivo. However, while CD4-positive cells obtained from CCR5-negative individuals are resistant to infection by viruses that require this coreceptor, they are readily infectable by viruses which use CXCR4 receptor [33,34]. In the literature we have not found any data on the influence of HIV infection on enzymes in saliva, except for those enzymes involved in innate immunity (lactoferrin, lysozyme, peroxidase) [35].

The aim of the present work was to evaluate the activity of lysosomal exoglycosidases in the saliva of HIV patients as indicators of glycoconjugate catabolism. Exoglycosidases [6] together with aminohydrolases and endoglycosidases take part in degradation of glycoconjugates [36]. Glycoconjugates are either proteins or lipids to which saccharide chains of different lengths are attached. The glycoconjugates (proteoglycans and glycolipids) function as receptors. Glycoproteins function both as receptors and transporters [37] on the surface of cellular membranes. Proteoglycans and glycoproteins are the main constituents of the intracellular matrix, where they form an intricate three-dimensional network responsible for proper hydratation, regulation of the activity of secreted proteins and exchange of the products of metabolism. Catabolism of glycoconjugates is connected with maintaining the balance between degradation of old and synthesis of new molecules. Exoglycosidases remove monosaccharides from the non-reducing end of oligo- or polysaccharide chains of glycoconjugates, by hydrolysis of glycosidic bonds.

We estimated the activity of N-acetyl-β-hexosaminidase and its isoenzymes (thermolabile isoenzyme A and thermostabile isoenzyme B), β-galactosidase, α-fucosidase and α-mannosidase, in the saliva of HIV infected patients. No significant differences were found between the activity of HEX and α-MAN in the saliva of HIV infected patients in comparison to control group. However, we noticed a significant increase in the activity of HEX-A, β-GAL and α-FUC, and a significant decrease in the activity of HEX-B in the saliva of HIV infected patients. Dramatical increase in GAL activity in saliva may be a result of intensive degradation of all glycoconjugates: glycoproteins, glycolipids and proteoglycans, as galactose is component of oligosaccharide chains of glycoproteins (especially salivary) [38], glycolipids [39] and glycosaminoglycans [40]. The lack of information in the literature concerning the activity of exoglycosidases in saliva of HIV infected patients, did not allow us any comparison of our results with data of other authors. Reports of increased activity of lysosomal exoglycosidases and salivary enzymes in the saliva of patients with periodontitis have been published [41-44]. In our study drug users HIV positive patients were included persons who did not care much about oral hygiene. We expected that an increase in the activity of lysosomal exoglycosidases could be found in HIV positive patients, because of presence of periodontal disease caused by poor oral hygiene. The lack of changes in the activity of HEX, the most characteristic enzyme in human tissue inflammation, could be explained by a low number of teeth and a low number of periodontal pockets. We conclude that within so low number of periodontal pockets even with existing inflammatory process differences in activity of salivary lysosomal exoglycosidases as compared to control could not be detected. The different number of teeth in groups is not very important in this study because volunteers included to control group (higher number of teeth) were healthy without periodontal disease. However, an increase in HEX-A activity as shown in our study, and in the light of previous research [10-12,45], can implicate the beginning of neoplastic changes developing in the oral cavity. An increase in GAL and FUC activity can implicate increase in catabolic process of glycoconjugates which is the sign of tissues destruction.

It has been reported that lymphocytes and macrophages are the sources of lysosomal exoglycosidases in saliva [43]. The way in which HIV infection changes the activity of exoglycosidases and the influence of the activity of exoglycosidases on HIV infection is still unknown. It is known, however, that the receptors for HIV are glycoproteins, but not which part of the HIV envelope oligosaccharide chains, if any, binds to the receptor on the surface of sensitive cells. It may be proposed that exoglycosidases removing appropriate sugars from non-reducing end of oligosaccharide chains can modify the possibility and strength of binding the envelope of HIV to cellular receptors, by exposing suitable oligosaccharide structures on the surface of sensitive cells. Thus the exoglycosidases can influence the docking of the HIV virus to the cell receptor.

HIV infection may induce T cell apoptosis through indirect mechanisms, including activation-induced cell death and autologous infected cell-mediated killing. The death of the cell by apoptosis or necrosis is preceded by the damage of cellular membranes. Lysosomal membranes also undergo this process

which result in release of their content to the cellular environment. Damage to lysosomal membranes of salivary glands may increase the release of exoglycosidases to saliva and change their activity. The release of the content of lysosomal granules to the extracellular matrix, crevicular fluid and saliva is responsible for destruction of periodontal tissue, associated with HIV infection [21,46,47]. The changes which we observed in the activity of lysosomal exoglycosidases in saliva from infected patients may result from any of the following causes:

– mutations of the sequences of DNA coding lysosomal exoglycosidases,

– disorders in biosynthesis of the polypeptide chains for lysosomal exoglycosidases,

– the influence of virus on chaperones which results in incorrect folding,

– degradation of exoglycosidases in endoplasmatic reticulum and Golgi apparatus,

– changes in activity of glycosylotransferases in membranes of endoplasmatic reticulum and Golgi apparatus damaged by HIV, which synthesis the oligosaccharide chains of lysosomal exoglycosidases. We have no data on concerning HIV influence on the activity or the structure of glycosyltransferases, or on the influence of HIV on the endoplasmatic reticulum and Golgi apparatus,

– disturbances of intracellular transport of exoglycosidases, by influence on the Man-6-P receptor or GGA proteins. This hypothesis is particularly interesting because HIV has affinity for the mannose receptor. HIV binding to the mannose receptor may block binding of Man-6-P of the oligosaccharide chain of lysosomal exoglycosidases to its receptor and this may trap exoglycosidases in the trans Golgi compartment.

Conclusion

Our results indicate that HIV and bacterial infections probably increase the catabolism of glycoconjugates in saliva by changing the relative activity of N-acetyl-β-glucosaminidase isoenzymes A and B as well as the activity of β-galactosidase and α-fucosidase.

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