Activity of lysosomal exoglycosidases in saliva of patients with HIV infection

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Abstract

Introduction: The aim of this work was to evaluate the influence of HIV infection on the catabolism of glycoconjugates in oral cavity, by determination the activity of lysosomal exoglycosidases in resting whole saliva HIV positive patients.

Material and methods: Sample of resting whole saliva from HIV infected patients (divided into two groups, depending on lymphocyte CD4+ number in peripheral blood) and the control-HIV negative group were analyzed for exoglycosidases activity. Determinations the activities (μKat/kg of protein) of lysosomal exoglycosidases were performed according to Chatteriee et al., modified Zwierz et al. The protein content (mg/ml) was determined by the Lowry method. Statistical analysis was performed using packet Statistica 6.0. Results were expressed as the mean and SD. P values less than 0.05 were considered significant.

Results: Exoglycosidases activities were not statistically dependent on immunological status of HIV patients. We obtained insignificant increase activities of HEX, HEX A and GALβ and insignificant decrease activity of HEX B along with the reduction of the CD4+ number. In both HIV positive groups the activities of HEX B were statistically lower and GALβ statistically higher in comparison to the control. In the case of HEX A significant differences could be observed between patients with low immunological status and the control group.

Conclusions: HIV infection intensifies catabolism glycoconiugates in saliva and changes activities of HEX, its isoenzymes A and B and β-galactosidase. It may change susceptibility the cells lining oral cavity to viral and bacterial infections.

Key words: HIV, lymphocyte CD4+, human saliva, lysosomal exoglycosidases.

Introduction

Glycoconiugates (glycoproteins, glycolipids and proteoglycans), form membranes of cells covering oral cavity, membranes on the teeth and intercellular substance of gingival's connective tissue [1]. Degradation the sugar moieties of glycoconjugates is performed by aminohydrolases, endoglycosidases and lysosomal exoglycosidases [2,3]. In normal saliva, the activity of lysosomal exoglycosidases is small, but sufficient to maintain steady state of glycoconjugates metabolism [4]. HIV infection can coexist with peridontopathy and neoplasm in oral cavity [5].

Chronic inflammation is accompanied by accumulation of neutrophiles, lymphocytes and mastocytes, which take part in destruction of the soft tissue of the oral cavity. Activity of proteolytic enzymes and enzymes degrading the glycoconiugates intensifies inflammatory changes and dystrophic digestion the host tissues in paradontium, and stimulates growth of microorganisms which participate in patomechanism of paradontitis, e.g. *Actinobacillus actinomycetemcomitans*, *Capnocytophag* [6].

The saliva is used to immunological and biochemical diagnosis of diseases of paradontium [7] with regard on easy accessibility and the possibility of non-invasive taking. Therefore the aim of the present work was evaluation the influence of the HIV infection on the catabolism of glycoconjugates in oral cavity, by determination the activity of lysosomal exoglycosidases in saliva of HIV positive patients.

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***Table 1*. Activity of lysosomal exoglycosidases in saliva of HIV positive patients and the control group**

Group	N	isoenzyme B μKat/kg protein		isoenzyme A μKat/kg protein		hexosaminidase (HEX) μKat/kg protein		β-galaktozydase (Gal) μKat/kg protein	
		mean	SD	mean	SD	mean	SD	mean	SD
I CD4+>500	12	7.54	1.55	8.95	1.33	16.49	1.48	2.03	0.108
II CD4+<499	37	7.09	0.58	10.25	0.62	17.35	1.03	2.04	0.09
III control	32	12.06	1.08	7.13	0.49	19.19	1.27	1.61	0.05
I:III		0.0229						0.0056	
p II:III		0.000288		0.000261				0.000041	

Material and methods

Consent of 49 HIV infected and 32 healthy persons (control – III) was obtained in accordance with guidelines of the Ethics Committee of Medical University of Białystok who approved the study (grant nr 3-70950L). HIV positive patients were hospitalized in Infectious Disease Clinic of Medical University in Białystok. These patients were divided into two groups: the Ist group – 12 persons CD4+>500/ml (I), the IInd group – 37 persons CD4+<500/ml (II).

3 ml of unstimulated, whole saliva was collected on ice by spitting method, under standardized conditions [8].

Salivary samples were centrifuged at 3000 x g for 20 minutes at 4°C to remove cells and debris [8]. The resulting supernatant was divided on 200 μl portions, frozen and kept at -80°C until analyzed.

Check-up of oral cavity was done in artificial light by use diagnostic dental tools. Condition of gingivae was evaluated by gingival index (GI) determined by the method Löe and Silness and papilla bleeding index (PBI).

Determinations the activities (μKat/kg of protein) of HEX, HEX A, HEX B and GALβ were performed according to Chatteriee et al. [9] in modification [3]. To 30 μl of substrate (p-nitrophenyl-N-acetyl-β-glucosaminide or p-nitrofenyl-β-D--galaktopiranozyde, Sigma) and 40 μl of 0.1M phosphate-citrate buffer, pH 4.7 or 4,3, 10 μl of diluted supernatant were added. Incubation time was 60 minutes at 37°C and reaction was stopped by adding 200 μl of 200 mM borate buffer, pH 9.8. Isoenzyme HEX A activity was calculated as the difference between the total activity of the enzyme and HEX B activity. The liberated p-nitrophenol was measured spectrophotometrically in a microplate reader, the Elx800™ at 405 nm. The protein content in supernatants (mg/ml) were measured according to the method of Lowry with BSA (Sigma) as a standard [10]. All determinations were performed in duplicate.

ANOVA followed by NIR test, and Spearman correlation were used for the statistical analysis. Results were expressed as the mean and SD. A level of $p \leq 0.05$ was considered to be significant.

Results

Results of this experiment are summarized in *Tab. 1*. One could conclude that HEX B activity in saliva of HIV-infected patients was not dependent on their immunological status and there was statistically lower in comparison to the control group (I and III $p<0.02$: II and III $p<0.0002$).

HEX A activity of infected patients was negatively correlated with the amount of lymphocyte CD4+ in peripheral blood. However, the significant differences could be observed between patients with low immunological status and the control group ($p<0.0002$).

HEX activity of infected patients increased with the decrease of the lymphocyte CD4+ number and it showed tendency to be lower in the comparison to the control.

GALβ activity in saliva of HIV positive groups was not changed with HIV progression and was statistically higher than in the control group (I and III $p<0.005$; II and III $p<0.00004$).

Discussion

It was reported, that HIV infects sensibility cells by binding to the receptor CD 4, and in case of lymphocytes deprived of CD4, through other receptors, e.g. the mannosidic or galactosidic type. The major determinant of viral tropism is at the level of entry. This occurs only if the appropriate coreceptor is present. Entry of HIV-I into its CD4+ target cells requires fusion/entry cofactors. Recently, the seven-transmembrane, G protein-coupled chemokine receptors CXCR4 and CCR5 were 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 [11,12]. CCR5 is the major coreceptor for HIV transmission *in vivo*. Except enzymes being part of innate immunity (lactoferrin, lysozyme, salivary peroxidase), in literature we did not find any data on influence of HIV infection on enzymes in saliva [13].

The aim of the present work was evaluation the activity of lysosomal exoglycosidases in saliva of HIV patients as indicators of glycoconjugates catabolism. Exoglycosidases [4] together with

aminohydrolases and endoglycosidases take part in degradation of glycoconjugates [2]. The glycoconjugates (glycoproteins, proteoglycans and glycolipids) are receptors, or transporters [14] on surface of cellular membranes. Catabolism of glycoconjugates is connected with maintaining balance between degradation of old and synthesis new molecules.

We estimated activity of N-acetyl-β-hexosaminidase, its isoenzymes (thermolabile isoenzyme A and themostabile isoenzyme B) and β-galactosidase in saliva of patients infected with HIV. The obtained results were analyzed depending on peripheral blood lymphocyte CD^{4+} level. Exoglycosidases activities were not statistically dependent on immunological status of HIV positive patients. We only could observed insignificant increase activities of HEX, HEX A and GAL β and insignificant decrease activity of HEX B along with the reduction of the CD^{4+} amount. Additionally in both HIV positive groups the activities of HEX B were statistically lower and GAL β statistically higher in comparison to the control. In the case of HEX A significant differences could be observed between patients with low immunological status and the control group. The lack of information in the literature on activity of exoglycosidases in saliva of HIV infected patients, did not permit on comparison our results with data of other authors. It was reported that lymphocytes and macrophages are source of lysosomal exoglycosidases in saliva [15]. There is unknown mechanism the influence of HIV infection on activity of exoglycosidases and influence the activity of exoglycosidases on HIV infection. It is known, that receptors for HIV are glycoproteins, but it is unknown if, and what part of oligosaccharide chains on HIV envelope binds to receptor on surface of sensitive cells. It should be supposed, 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 the exposure of suitable oligosaccharide structures on surface of sensitive cells. Thus the exoglycosidases can influence the docking HIV to the cell receptor.

HIV infection is associated with enhanced apoptosis in CD4 T cells infected by HIV and in uninfected T cells. Death of the cell by apoptosis or necrosis is preceded be the damage of cellular membranes, and lysosomal included, and liberation their content. Damage to lysosomal membranes of salivary glands may increase liberation of exoglycosidases to saliva and change their activities. Release the content of lysosomal granules to the extracellular matrix and saliva is responsible for inflammatory state in oral cavity associated with HIV infection [6]. The observed by us changes in activity of lysosomal exoglycosidases in saliva of infected patients may result from: the mutations the sequences of DNA coding lysosomal exoglycosidases, the disorders in biosynthesis the polypeptide chains for lysosomal exoglycosidases, influence of virus on chaperones, changes in activity glycosylotransferases damage by HIV membranes of endoplasmatic reticulum and Golgi apparatus, which synthesize the oligosaccharic chains of lysosomal exoglycosidases, disturbances of intracellular transport of exoglycosidases, by influence on Man-6-P receptors or GGA proteins. This later hypothesis is particularly interesting because HIV has affinity toward mannosidic receptor. HIV binding to the mannosidic receptor may block binding Man-6-P of the oligosaccharide chain of lysosomal exoglycosidases with their receptor, and it stops exoglycosidase in trans Golgi compartment, or export outside the cell with omission of lysosomes.

Conclusions

Infection by HIV intensifies catabolism glycoconiugates in saliva and changes the activities of HEX, its isoenzymes A and B and β-galactosidase. It can be a reason of changes in susceptibility the cells lining oral cavity to viral and bacterial infections.

Abbreviation

HEX-N – acetyl-β-hexosaminidase
HEX A – isoenzyme A of N-acetyl-β-hexosaminidase
HEX B – isoenzyme B of N-acetyl-β-hexosaminidase
GAL β – β-galactosidase

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