

N-Acetyl-Beta-Hexosaminidase Activity in Asthma

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Key Words

N-acetyl- β -hexosaminidase · Asthma · Remodeling · Airway inflammation

Abstract

Background: N-acetyl- β -hexosaminidase (β -hex) is a lysosomal hydrolase, which is selectively secreted into the extracellular space by inflammatory cells. The aim of our study was to assess the activity of β -hex in the plasma of asthmatic patients, and to establish whether it correlates with asthma severity and airway inflammation. **Methods:** The study was conducted in a group of 46 asthmatic patients and 13 healthy volunteers. All study participants underwent analysis of exhaled nitric oxide and flow-volume spirometry. β -hex activity, peripheral blood eosinophils, total serum IgE and eosinophil cationic protein were analyzed in blood samples from all asthmatic patients and healthy volunteers. **Results:** β -hex activity was significantly higher in patients with severe or moderate asthma compared with healthy volunteers and was positively correlated with exhaled nitric oxide levels and serum eosinophil cationic protein in these groups of patients. There was no correlation between β -hex activity and forced expiratory volume in 1 s, blood eosinophil count or total serum IgE in these groups of asthmatics. **Conclusions:** Our results suggest that β -hex could take part in airway inflammation and remodeling in asthma. Our study is the first report in which the elevated activity of β -hex in sub-

jects with asthma has been observed. However, more studies are needed to establish the precise role of this enzyme in asthma in humans.

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Introduction

Asthma is a chronic inflammatory disease of the airways in which many cell types play a role – in particular mast cells, eosinophils and T lymphocytes [1]. Airway remodeling is a special feature of asthma, in which airway smooth muscle proliferation occurs as a consequence of inflammation [2]. Thus, asthma should be seen as a continuum from symptoms to airway wall remodeling, though the mechanisms underlying this process remain unclear [3].

Upon activation, mast cells and eosinophils release to their surroundings numerous substances serving as mediators of extracellular matrix and mitogenesis [4, 5]. The majority of these substances are stored and released from highly specialized granules present in such cells [4, 5]. The secretory granules are believed to have a lysosomal origin, based on the assumption that the granules are also expected to contain and release acid hydrolases, ubiquitous in lysosomes [6]. Although the characteristics and physiological role of specific substances stored in mast cells and eosinophil granules have been extensively stud-

ied in asthmatic patients, little is known about the involvement of classical lysosomal acid hydrolases in the pathophysiology of this disease. One such hydrolase is N-acetyl- β -hexosaminidase (β -hex). This is a proteolytic enzyme which releases N-acetyl-glucosamine and N-acetyl-galactosamine from the nonreducing end of oligosaccharide chains of glycoproteins, glycolipids and glycosaminoglycans [7]. Glycosaminoglycans are important substrates for β -hex [8]. β -hex is a lysosomal hydrolase which is secreted into the extracellular space selectively by inflammatory cells such as mast cells, basophils, macrophages, eosinophils and neutrophils [9–11].

Little is known about classical lysosomes containing such acid hydrolases, including β -hex, in asthma. Therefore, the aim of this study was to assess β -hex activity in asthmatic patients with different degrees of severity of the disease and to establish a possible correlation between the activity of this enzyme and other parameters used for assessment of asthma severity and airway inflammation.

Material and Methods

Patients

The study involved a group of 46 patients with mild to severe allergic asthma (15 with mild, 10 with moderate and 21 with severe asthma). Asthma was diagnosed according to the criteria recommended by the Global Initiative for Asthma (GINA) [1]. All patients were in a stable condition, free from acute exacerbations and respiratory tract infections during the previous 2 months. Patients who presented other factors that could alter exhaled nitric oxide (F_{ENO} ; except for asthma, features of atopy or allergic rhinitis) were excluded from the study. F_{ENO} measurement and flow-volume spirometry were performed in each asthmatic patient. The presence of allergy to common allergens (house dust mite, trees, weeds, grasses, cat, *Alternaria* or *Cladosporium*) was demonstrated in all patients by positive skin prick tests. All patients had been treated with stable doses of inhaled glucocorticosteroids during the past 3 months: patients with mild asthma received a daily dose of 200–400 μ g budesonide or its equivalent, patients with moderate asthma a daily dose of >400–800 μ g budesonide or its equivalent and patients with severe asthma a daily dose of >800–1,600 μ g budesonide or its equivalent, according to GINA 2002 [1]. Patients treated with oral glucocorticosteroids were excluded from the study.

Thirteen healthy volunteers were used as a negative control group. All of them underwent analysis of F_{ENO} and flow-volume spirometry. They were free of respiratory tract infection for 2 months prior to the study and from other significant illnesses known to affect F_{ENO} measurements.

β -hex activity, peripheral blood eosinophils, total serum IgE and eosinophil cationic protein (ECP) were analyzed in blood samples from all asthmatic patients and healthy volunteers.

Asthmatic patients and healthy volunteers were nonsmokers and had not been passive smokers in the previous year.

The study protocol was approved by the research Ethics Committee of the Medical University of Białystok (reference No. R-I-003/94/2006). Informed consent was obtained from all patients enrolled in the study.

Measurements

F_{ENO} was measured in all asthmatic patients and healthy subjects using the chemiluminescence technique with a Sievers 280i nitric oxide (NO) analyzer (Sievers, USA). The measurements were performed at an expiratory flow of 50 ml/s [12]. The duration of exhalation had to be at least 6 s to produce a stable NO level for 3 s. Three recorded F_{ENO} measurements were obtained for each subject. Repeat measurements were performed until the 3 values agreed to within 10% of the mean. The mean value of the 3 measurements was recorded as the final F_{ENO} .

Baseline spirometry was performed using a MasterScreen Pneumo PC spirometer (Jaeger, Germany). Spirometry was performed according to American Thoracic Society guidelines [13]. Forced expiratory volume in 1 s (FEV_1) was evaluated. The highest value from 3 technically satisfactory attempts was attached.

Blood eosinophil count was measured using a hematologic analyzer (Coulter Electronics GmbH, Germany). Total serum IgE concentration and serum ECP concentration were measured by immunoassay using the ImmunoCAPTM system (Pharmacia Diagnostics, Sweden).

β -hex Activity Measurement

Blood Collection

Blood samples were collected after an overnight fasting period, into tubes containing 3.2% sodium citrate (10:1 vol/vol); platelet counts were measured using a Coulter STKS. Platelet-rich plasma was prepared by centrifugation of whole blood at 200 g for 15 min at room temperature; platelet-poor plasma was obtained by centrifuging platelet-rich plasma at 2,000 g for 6 min, and platelet-filtrated plasma (PFP) was obtained by centrifuging platelet-poor plasma at 11,000 g for 10 min.

Determination of β -hex Activity

β -hex (EC 3.2.1.52) activity was determined in freshly prepared PFP samples as described previously [14].

Briefly, PFP samples (50 μ l) were supplemented with 50 μ l of 5 mM *p*-nitrophenyl-*N*-acetyl- β -D-glucosamide in 0.1 M citrate phosphate buffer, pH 4.5, at 37°C for 60 min. At the end of the incubation, 200 μ l 0.1 M NaOH was added. Absorbance was monitored at 405 nm. Results are expressed in micromoles of released *p*-nitrophenyl per liter of PFP. The activity of β -hex was expressed in international units (IU) as the number of micromoles of *p*-nitrophenyl liberated during 1 min and was calculated for 1 liter of PFP.

Statistical Analysis

Data were compared using one-way analysis of variance followed by Bonferroni's *t* test post hoc to determine statistical differences. All values were expressed as means $\pm$ SD. The relationship between studied parameters was assessed using Pearson's linear correlation coefficient. $p < 0.05$ were considered statistically significant.

Table 1. Characteristics of studied asthmatic patients and healthy volunteers

	Healthy volunteers	Mild asthma	Moderate asthma	Severe asthma
Patients, n	13	15	10	21
Sex, F/M	8/5	9/6	7/3	16/5
Age, years	36.7 ± 8.0	39.5 ± 9.9	45.6 ± 10.3	48.4 ± 8.3
β-hex activity, IU/l	2.66 ± 0.78	3.63 ± 1.69	3.97 ± 1.03 ^a	6.39 ± 1.90 ^{a, b, c}
Baseline FEV ₁ , % predicted	97.5 ± 8.6	96.5 ± 11.7	77.1 ± 15.0 ^{a, b}	49.5 ± 8.9 ^{a, b, c}
Blood eosinophil count, cells/mm ³	88.5 ± 40.5	159.7 ± 121.4	103.2 ± 75.8	147.8 ± 140.5
Serum concentration of ECP, µg/l	3.16 ± 0.9	6.8 ± 1.6 ^a	9.9 ± 2.2 ^{a, b}	10.3 ± 3.7 ^{a, b}
Serum total IgE concentration, kU/l	66.1 ± 48.9	192.3 ± 146.5 ^a	101.5 ± 60.7 ^a	180.4 ± 133.3 ^a
F _{ENO} , ppb	15.5 ± 5.9	62.5 ± 45.4 ^a	132.5 ± 83.9 ^{a, b}	54.3 ± 32.5 ^{a, c}

Data are presented as means ± SD. ^a $p < 0.05$ versus healthy volunteers; ^b $p < 0.05$ versus mild asthma; ^c $p < 0.05$ versus moderate asthma.

Results

The characteristics of the patients and healthy volunteers are presented in table 1. The highest levels of β-hex activity were observed in patients with severe asthma. This activity was statistically significantly higher compared with patients with mild or moderate asthma or healthy volunteers. In moderate asthmatics, statistically significant increased β-hex activity was observed compared with healthy volunteers. In this group of asthmatic patients, the activity of this enzyme was higher than in mild asthmatics, but these differences were not statistically significant. Comparing patients with mild asthma with healthy volunteers, higher β-hex activity was observed in mild asthmatics; however, these differences were not statistically significant. These results are presented in figure 1.

We observed that β-hex activity increased with asthma severity ($p < 0.01$). The relationship between β-hex activity and age of patients within each studied group did not reveal statistically significant correlations. There was also no correlation between activity of β-hex in females versus males in all groups studied.

In the groups of severe and moderate asthmatics, β-hex activity was positively correlated with F_{ENO} levels and serum ECP. There were no correlations between β-hex activity and FEV₁, blood eosinophil count or total serum IgE in these groups of patients.

In the groups of mild asthmatics and healthy volunteers, there were no correlations between β-hex activity and F_{ENO} levels, serum ECP, FEV₁, blood eosinophil count or total serum IgE. The correlations between β-hex activity and other studied parameters in all groups of asthmatics and healthy volunteers are presented in table 2.

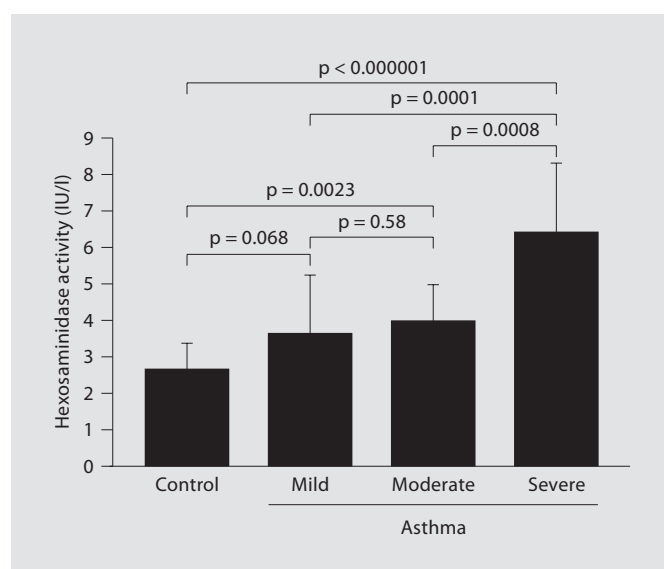


Fig. 1. β-hex activity in healthy volunteers and particular groups of asthmatics.

Comparisons of the studied parameters (F_{ENO}, FEV₁, ECP, total IgE and blood eosinophilia) in particular groups of asthmatics and healthy volunteers are presented in table 3.

Discussion

There is no information about β-hex activity in humans with asthma. Therefore, the aim of our study was to assess β-hex activity in asthmatic patients and healthy

Table 2. Correlations of β -hex activity with other studied parameters

Study groups	F_{ENO}		ECP		Blood eosinophilia		Total IgE		FEV_1	
	r	p	r	p	r	p	r	p	r	p
Healthy controls	0.0002	0.99	0.1044	0.28	0.0008	0.93	0.0865	0.33	0.0304	0.57
Mild asthma	0.0299	0.54	0.0377	0.49	0.0097	0.73	0.0844	0.29	0.0179	0.63
Moderate asthma	0.5644	0.01	0.4546	0.03	0.0298	0.63	0.0317	0.62	0.0021	0.90
Severe asthma	0.6245	<0.01	0.4453	<0.01	0.0069	0.72	0.0678	0.25	0.0068	0.72

Table 3. Comparison of studied parameters between healthy volunteers and particular groups of asthmatics

	p value				
	F_{ENO}	ECP	blood eosinophilia	total IgE	FEV_1
Healthy controls versus severe asthma	<0.01	<0.01	0.15	<0.01	<0.01
Healthy controls versus moderate asthma	<0.01	<0.01	0.55	0.13	<0.01
Healthy controls versus mild asthma	<0.01	<0.01	0.05	<0.01	0.78
Severe asthma versus moderate asthma	<0.01	0.77	0.35	0.08	<0.01
Severe asthma versus mild asthma	0.53	<0.01	0.79	0.8	<0.01
Moderate asthma versus mild asthma	0.01	<0.01	0.2	0.07	0.01

volunteers and to establish a possible correlation between the activity of this enzyme and other parameters used for assessment of asthma severity and airway inflammation. We observed that the activity of this enzyme is elevated in patients with asthma compared with healthy volunteers as well as with asthma severity.

What is the source of β -hex in asthma? β -hex is released from all cells containing lysosomes, including mast cells, basophils, macrophages, eosinophils and neutrophils [9–11]. All these cells are activated during asthmatic inflammation [15].

Many studies have demonstrated that increased serum activity of β -hex is a consequence of the release of this enzyme from restricted areas in the human body, such as neoplasms or liver cells [16–18]. Plucinsky et al. [19] have shown that in patients with colorectal cancer β -hex serum activity is increased and elevated levels of this enzyme have been observed in malignant tissue compared to the activity seen in uninvolved normal colon tissue. Therefore, the plasma level of β -hex could be a reflection of many physiological and pathological processes, limited to particular cells, tissues or organs.

We observed that in the groups of moderate and severe asthmatics β -hex activity was correlated well with ECP, which is the marker of eosinophil activation [20], as well

as with F_{ENO} , which is in turn produced by many inflammatory cells such as macrophages, eosinophils and airway epithelial cells [15]. Thus, the increase in plasma β -hex activity in asthma could be a consequence of the activation of all these cells during airway inflammation.

Gabazza et al. [21] suggest that NO plays a key role in the pathogenesis of airway remodeling. NO can modulate airway smooth muscle tone, bronchial edema and bronchial inflammation, and is known to be increased in the exhaled air of asthmatic patients [22, 23]. We observed that β -hex activity correlates well with F_{ENO} levels in patients with moderate or severe asthma. Therefore, we suggest that this enzyme could participate in airway inflammation and remodeling. β -hex and other mannosyl-rich lysosomal hydrolases are potent mitogens for bovine airway smooth muscle cells [24], and this mitogenic action is mediated via airway smooth muscle mannose-recognizing receptors [25] that have been isolated from bovine airway smooth muscle cells and human bronchial smooth muscle [26]. Lew et al. [27] demonstrated that β -hex-induced airway smooth muscle cell proliferation is mediated by activation of p21Ras/MEK/p44/42^{MAPK} and protein kinase C. β -hex causes activation of p44/42^{MAPK} that is late in onset (30 min) and sustained for 4 h [27]. The pattern of β -hex-induced p44/42^{MAPK} is probably

critical to cell proliferation [28–30]. Moreover, β -hex is protease resistant [26] and, upon activation, first degrades the extracellular matrix and subsequently acts as a mitogenic factor [27]. Thus, β -hex may serve as a key inflammatory mediator critical to the airway remodeling process [26] and the mitogenic activity of this enzyme in humans is very likely; however, more studies are needed to establish its precise role in airway remodeling.

Studies on animal models have revealed that β -hex activity is associated with mitogenesis and airway remodeling [24, 27]. Our studies demonstrate that β -hex activity is statistically significantly higher in patients with mod-

erate or severe asthma than in healthy persons. These results suggest that β -hex may contribute to inflammation and airway remodeling in asthma. However, more studies are needed to establish the precise role of this enzyme in the pathogenesis of this disease.

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References

- 1 Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention. NIH Publication No. 02-3659. Bethesda, National Heart, Lung and Blood Institute, 2002.
- 2 Dixon ER, Weinberg JA, Lew DB: Effect of dexamethasone on bovine airway smooth muscle cell proliferation. *J Asthma* 1999;36: 519–525.
- 3 Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM: State of the art: asthma. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 2000;161:1720–1745.
- 4 Weller PF: The immunobiology of eosinophils. *N Engl J Med* 1991;324:1110–1118.
- 5 Black J: The role of mast cells in the pathophysiology of asthma. *N Engl J Med* 2002; 346:1742–1743.
- 6 Blott EJ, Griffiths GM: Secretory lysosomes. *Nat Rev Mol Cell Biol* 2002;3:122–131.
- 7 Zwierz K, Juszkievicz J, Arciuch L, Gindziński A: N-acetyl- β -hexosaminidase – the enzyme of Tay-Sachs and Sandhoff diseases (in Polish). *Postepy Biochem* 1992;38: 127–132.
- 8 Suzuki K, Sango K, Proia RL, Langaman C: Mice deficient of all forms of lysosomal β -hexosaminidase show mucopolysaccharidosis-like pathology. *J Neuropathol Exp Neurol* 1997;56:693–703.
- 9 Torigoe C, Inman JK, Metzger H: An unusual mechanism for ligand antagonism. *Science* 1998;281:568–572.
- 10 Schnyder J, Baggiolini M: Secretion of lysosomal hydrolases by stimulated and non-stimulated macrophages. *J Exp Med* 1978; 148:435–450.
- 11 Lew DB, Leslie CC, Riches DWH, Henson PM: Induction of macrophage lysosomal hydrolase by synthesis and secretion by β -1,3-glucan. *Cell Immunol* 1986;100:340–350.
- 12 Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. *Am J Respir Crit Care Med* 1999;160:2104–2117.
- 13 Lung function testing: selection of reference values and interpretative strategies. American Thoracic Society. *Am Rev Respir Dis* 1991;144:1202–1218.
- 14 Rohrer J, Schweizer A, Johnson KF, Kornfeld S: A Determinant in the cytoplasmic tail of the cation-dependent mannose 6-phosphate receptor prevents trafficking to lysosomes. *J Cell Biol* 1995;130:1297–1306.
- 15 Barnes PJ, Fan Chung K, Page CP: Inflammatory mediators of asthma: an update. *Pharmacol Rev* 1998;50:515–596.
- 16 Lo CH, Kritchevsky D: Human serum hexosaminidases: elevated B form isoenzyme in cancer patients. *J Med* 1978;9:313–336.
- 17 Brattain MG, Kimball PM, Pretlow TG: β -Hexosaminidase isoenzymes in human colonic carcinoma. *Cancer Res* 1977;37:731–735.
- 18 Hultberg B, Isaaksson A: Isoenzyme pattern of serum β -hexosaminidase in liver disease, alcohol intoxication and pregnancy. *Enzyme* 1983;30:166–171.
- 19 Plucinsky MC, Prorok JJ, Alhadeff JA: β -Hexosaminidase from colon and sera of dukes-classified colorectal cancer patients: activity levels, isoenzyme patterns, and kinetic properties. *J Natl Cancer Inst* 1986;77: 57–62.
- 20 Koh GC, Shek LP, Goh DY, Van Bever H, Koh DS: Eosinophil cationic protein: is it useful in asthma? A systematic review. *Respir Med* 2007;101:696–705.
- 21 Gabazza EC, Taguchi O, Tamaki S, Murashima S, Kobayashi H, Yasui H, Kobayashi T, Hataji O, Adachi Y: Role of nitric oxide in airway remodeling. *Clin Sci* 2000;98:291–294.
- 22 Robbins RA: Nitric oxide; in Barnes PJ, Grunstein MM, Leff AR, Woolcock AJ (eds): *Asthma*. Philadelphia, Lippincott-Raven, 1997, pp 27–32.
- 23 Kharitonov SA, Yates D, Robbins RA: Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994;343:133–135.
- 24 Lew DB, Rattazzi MC: Mitogenic effect of lysosomal hydrolases on bovine tracheal myocytes in culture. *J Clin Invest* 1991;8:1969–1975.
- 25 Lew DB, Songu-Mize E, Pontow SE, Stahl PD, Rattazzi MC: A mannose receptor mediates mannosyl-rich glycoprotein-induced mitogenesis in bovine airway smooth muscle cells. *J Clin Invest* 1994;94:1855–1863.
- 26 Lew DB: β -Hexosaminidases: potent mitogens of airway smooth muscle. *Allergy Asthma Proc* 1998;19:359–363.
- 27 Lew DB, Dempsey BK, Zhao Y, Muthalif M, Fatima S, Malik KU: β -hexosaminidase-induced activation of p44/42 mitogen-activated protein kinase is dependent on p21Ras and protein kinase C and mediates bovine airway smooth-muscle proliferation. *Am J Respir Cell Mol Biol* 1999;21:111–118.
- 28 Johnson GL, Vaillancourt RR: Sequential protein kinase reactions controlling cell growth and differentiation. *Curr Opin Cell Biol* 1994;6:230–238.
- 29 Meloche S, Seuwen K, Pages G, Pouyssegur J: Biphasic and synergistic activation of p44mapk (ERK1) by growth factors: correlation between late phase activation and mitogenicity. *Mol Endocrinol* 1992;6:845–854.
- 30 Marshall CJ: Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell* 1995;80:179–185.