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Activity of N-acetyl-β-hexosaminidase and its isoenzymes in serum and synovial fluid from patients with different arthropathies

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ABSTRACT

Objective. *To evaluate the activity of N-acetyl-β-hexosaminidase (HEX) and its isoenzymes in the serum and synovial fluid of healthy volunteers and patients with an injury to the anterior cruciate ligament and/or meniscus (ACL) osteoarthritis (OA), juvenile idiopathic arthritis (JIA) and rheumatoid arthritis (RA).*

Methods. *The activity of HEX and its isoenzymes was determined according to Zwierz* et al. *method. Protein content was determined by the biuret method.*

Results. *The specific activity of HEX and its isoenzymes in the serum of patients with JIA showed a tendency to increase in comparison to the reference group. The specific activity of total HEX in the serum of RA patients was significantly increased in comparison to control. Our results show, that specific activity of HEX in synovial fluid, in the reference group 4.2 ± 0.21 μkat/kg protein (0.25 unit/mg protein), is similar to activity in normal temporomandibular joint fluid (0.3 unit/mg protein). Therefore, we included this group in our research. In patients with OA and ACL injuries, HEX and its isoenzymes showed a tendency to increase in the specific activity in synovial fluid. The specific activity of HEX and its isoenzymes in the synovial fluid of patients with RA and JIA was significantly elevated in comparison to the control and the remaining groups.*

Conclusion. *In the synovial fluid of patients with JIA and RA, the specific activity of HEX and its isoenzymes significantly increased in comparison to control and patients with diseases of a non-inflammatory etiology (OA and ACL). In the synovial fluid of control and diseased groups, HEX constituted a higher percent of total proteins than in serum.*

Introduction

In healthy joints there is a balance between the biosynthesis and degradation of proteoglycans and glycosaminoglycans in articular cartilage and synovial fluid. The articular cartilage of patients with arthropaties has a reduced amount of extracellular matrix proteoglycans (1) and decreased size of proteoglycans and glycosaminoglycans (2). Reduction in the amount of hyaluronic acid and other glycosaminoglycans in the synovial fluid causes weaker lubrication and a loss of the biomechanical properties of affected joints (3). Degradation of the cartilage matrix and synovial fluid proteoglycans and glycosaminoglycans is performed by the concerted action of proteases (4) and glycosidases (5). Most scientists believe that metaloproteinases e.g. collagenases, stromelysins, aggrecanase and cysteine proteases (6), play a main role in the destruction of articular cartilage, by degrading cartilage proteins. However, oligo and polysaccharide chains of glycoproteins, and glycosaminoglycans of cells, extra cellular matrix, and synovial fluid, are degraded by glycosidases (7, 8). N-acetyl-β-hexosaminidase (HEX) is the most active enzyme of the exoglycosidases (9).

There is little information on the activity of exoglycosidases in healthy joints and only a few reports on the activity of exoglycosidases in diseased joints (7, 10) and serum (11). The aim of the present paper is to evaluate the specific activity of HEX and its isoenzymes in the serum and synovial fluid of healthy people and of patients with the knee injury (ACL), osteoarthritis (OA), juvenile idiopathic arthritis (JIA) and rheumatoid arthritis (RA).

Materials and methods

Patients

Samples of serum and synovial fluid were obtained from staff and patients of the Medical University of Bialystok as follows:

- 10 healthy volunteers (6 women, 4 men, age range 24-62 years) with no history of injuries or joint disease.
- 30 patients (10 women, 20 men, age range 17-21) from the Department of Paediatric Orthopedics and Traumatology, with arthroscopically verified injury to the anterior cruciate ligament and/or meniscus of the knee from 3 to 27 months after the trauma.
- 20 patients (12 women and 8 men) from the Department of Rheumatol-

ogy with primary medium gravidity knee OA, aged 46-72 years, fulfilling the clinical and radiological criteria of the American College of Rheumatology (12). Patients with knee OA all had radiological evidence of narrowing of joint space and osteophyte in one or more knee compartments.

- 15 patients (5 girls and 10 boys, age range 6-16 years) from the II Department of Paediatrics, with JIA. Patients were classified according to the International League of Associations for Rheumatology (ILAR) classification criteria (13). All patients with JIA in this study were Polish Caucasian.
- 15 patients (10 women and 5 men, age range 22-74) from the Department of Rheumatology, with RA (according to the 1987 year criteria of the American College of Rheumatology – formerly the American Rheumatism Association) (14), who had a clinically inflamed knee joint with effusion, joint swelling, and pain.

Consent of patients and healthy persons was obtained in accordance with the guidelines of the Ethics Committee of the Medical University of Bialystok who approved the study (R-I-003/338/2003).

Synovial fluid and serum samples
Preparation of synovial fluid from:

- healthy volunteers – a solution of lidocaine (1%) was injected subcutaneously (0.5ml) and intraarticulary (1ml) to the knee. Synovial fluid mixed with the lidocaine solution was aspirated utilizing a lateral infrapatellar approach, with a 6 mm needle.
- patients with OA, JIA, RA – synovial fluid was taken similarly as from healthy volunteers except for injecting lidocaine into the joint.

Samples were collected in sterile tubes and centrifuged at 10.000 g for 15 minutes at 4˚C to remove cells and cell debris. The supernatants were stored at -80˚C until biochemical assay.

Preparation of serum:

- Two ml samples of blood were collected in sterile tubes, allowed to clot and centrifuged at 5.000 g for 5 minutes at 4˚C. Samples of serum were stored at -80˚C until assay.

Biochemical assessment

The specific activity of N-acetyl-β-hexosaminidase (E.C.32.1.52) in samples of the synovial fluids and serum was determined by the method described by Zwierz *et al.* (8). To 30 μl of substrate (p-nitrophenyl-N-acetyl-β-glucosaminide, Sigma) and 40 μl of 0.1*M* phosphate-citrate buffer, pH 4.7, 10 μl of diluted sample were added. Incubation time was 60 minutes at 37˚C and reaction was stopped by adding 200 μl of 200 m*M* 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. Protein content was determined by the biuret method with bovine serum albumin as a standard (15). The liberated p-nitrophenol was measured spectrophotometrically in a microplate reader, the Elx800™ at 405 nm.

Statistical analysis

Statistical analysis was performed with a Statsoft program by Statistica 5.0. Results were expressed as the mean and SD. P values of less than 0.05 were considered significant.

Results

The specific activity of N-acetyl-β-hexosaminidase and its isoenzyme A in serum of patients with RA: (4.0 ± 1.8 μkat/kg of protein) and (1.86 ± 0.8 μkat/kg of protein), respectively, significantly increased in comparison to patients with ACL injury, (2.54 ± 0.71 μkat/kg of protein) and (1.1 ± 0.4 μkat/kg of protein) respectively, and to the reference group of volunteers with healthy knees. The activity of HEX and its isoenzyme A in the group of patients with JIA was significantly increased in comparison to the ACL group (3.4 ± 1.0 μkat/kg of protein), (1.8 ± 0.6 μkat/kg of protein) and (2.54 ± 0.71 μkat/kg of protein), (1.1 ± 0.4 μkat/kg of protein) respectively. The activity of HEX and its isoenzymes in the group of patients with OA tended to increase in comparison to the control and ACL groups. We observed an increase in the activity of isoenzyme A over that of isoenzyme B, in the groups of patients with RA, JIA and OA.

There was a significant increase in the specific activity of HEX and its isoenzymes in the synovial fluid (Table I) of patients with RA and JIA in comparison to groups with OA, ACL, and control. There was a pronounced trend of increase in the specific activity of HEX and its isoenzymes in the OA and ACL groups in comparison to control, but the increase was not significant.

Discussion

In clinical research there is usually the problem of a reference group for comparison with the results obtained in diseased patients. Data on the HEX activity in normal, human, temporomandibular joint (TMJ) synovial fluid has been reported in the literature (16), but no data could be found on the activity of N-acetyl-β-hexosaminidase in the knee joint synovial fluid of healthy persons. Our results show that specific activity of HEX in knee joint synovial fluid in our reference group (4.2 ± 0.21 μkat/kg protein = 0.25 unit/mg protein) is similar to the activity in a normal TMJ joint fluid (0.3 unit/mg protein) reported in the literature (16).

The specific activity of N-acetyl-β-hexosaminidase and its isoenzymes in the serum and synovial fluid in the reference group of 10 volunteers with healthy knees (control group) and of patients with ACL, OA, JIA and RA, is presented in TableI. The specific activity of HEX in the synovial fluid of the control group is 1.55 times higher than in the serum. We have found that in JIA and RA patients, the specific activity of HEX in synovial fluid is even 6-8 times higher than in serum. The above results suggest that HEX in synovial fluid derives mainly from articular tissues or articular leucocytes (5) and not from serum. Therefore, determining HEX in synovial fluid better reflects the situation in the joint cavity than determining HEX in serum. Our results are in agreement with the results of Ortutay *et al.* (17) which suggest the activity of exoglycosidases in synovial fluid as a predictor of RA and cartilage glycosaminoglycan depletion.

Table I. Specific activity of N-acetyl-β-hexosaminidase and its isoenzymes in serum and synovial fluid (μkat/kg of protein).

Patients	HEX Activity in		IZO A Activity in		IZO B Activity in	
	Serum	SF	Serum	SF	Serum	SF
(I) CONTROL N = 10	2.71 ± 0.52	4.2 ± 0.21	1.31 ± 0.3	2.3 ± 1.41	1.4 ± 0.44	2.1 ± 1.37
(II) ACL injury N = 30	2.54 ± 0.71	5.16 ± 2.13 ↑ 22.8%	1.1 ± 0.4	2.4 ± 1.25 ↑ 4.4%	1.46 ± 0.44	2.76 ± 1.46 ↑ 3.4%
(III) OA N = 20	3.18 ± 1.93 ↑ 17.3%	5.33 ± 3.28 ↑ 26.9%	1.57 ± 1.14	2.78 ± 2.3 ↑ 20.87%	1.62 ± 0.8	2.56 ± 1.7 ↑ 22.0%
(IV) JIA N = 15	3.4 ± 1.0 ↑ 25.46%	29.86 ± 16.9 ↑ 610.9%	1.8 ± 0.6 ↑ 37.4%	14.8 ± 7.8 ↑ 543.4%	1.6 ± 0.6 ↑ 7.14%	13.2 ± 9.5 ↑ 528.5%
(V) RA N = 15	4.0 ± 1.8 ↑ 47.6%	26.67 ± 11.5 ↑ 535%	1.86 ± 0.8 ↑ 37.4%	12.3 ± 7.86 ↑ 444.8%	2.07 ± 1.6 ↑ 35.7%	11.9 ± 6.0 ↑ 464.8%
P	IV:II p = 0, 03888 V:I p = 0,000602 V:II p = 0,000287	IV: I; II; III p = 0,00088 V: I; II; III p = 0,00088	IV:II p = 0,005857 V:I p = 0,005857 V:II p = 0,000632	IV: I; II; III p = 0,00088 V: I; II; III p = 0,00088	value not significant	V: I; II; III p = 0,00088 V: I; II; III p = 0,00088

*↑% increases in comparison to control.

In the synovial fluid of JIA and RA patients, we found a significant increase in the specific activity of HEX and its isoenzymes in comparison to HEX activity in synovial fluid from OA and ACL injuries. We have previously reported an increase in the specific activity of HEX in synovial fluid, connected with the time that has passed from the injury i.e. after damage to the crucial ligament of the knee (7). This suggests that damage to the joint stimulates articular tissues to increased secretion of HEX and its isoenzymes, as a reaction on the remodeling of the tissues. A significant increase in activity of HEX and its isoenzymes in JIA and RA synovial fluid, in comparison to OA and ACL injuries, suggests that release of HEX to synovial fluid is greatly enhanced by the autoimmunological inflammatory process in knee joint cavity. Our results suggesting elevated lysosomal HEX associated with the progression of rheumatoid arthritis are in agreement with results on the activity of lysosomal enzymes in RA leucocytes reported by Sohar *et al*. (5).

In the serum of RA patients, the specific activity of HEX was significantly increased in comparison to control (Table I), which is in agreement with data reported by Berenbaum *et al*. (10). The increase of HEX activity in the serum of RA patients may depend on the increase in HEX activity of RA leucocytes as reported Sohar *et al.* (5). Our results may support this suggestion as, in the serum of patients with JIA and OA, we observed a moderate increase in HEX activity, 25.46 and 17.3% respectively. In the case of patients with ACL injury, the specific activity of HEX and its isoenzymes in serum behaved similarly as in the control.

Human hexosaminidase has two main isoenzymes: HEX A (subunits α, β) and HEX B (subunits β, β). HEX S (subunits α, α) is of minor importance, as it constitutes less than 0.02% of HEX A activity (18). We have found a high activity of both isoenzymes A and B in serum and in SF patients with OA, JIA, and RA, with HEX A representing an average of 48%of total HEX activity in serum and 52% of total HEX activity in synovial fluid. Our results are not consistent with the results of Gelger *et al.* (19) who suggest that in serum and synovial fluid isoenzyme A dominates (80% of total HEX). In our experience, estimation of the relative activity of HEX isoenzymes depends on the methods used for their determination, which suggests that only total HEX measurement has practical value. Our data indicate that in the synovial fluid of patients with RA and JIA, the activity of HEX is several times higher in comparison to control and to patients with joint diseases without inflammation (OA and ACL). The above data suggest that in joints with rheumatoid inflammation there is an increase in HEX production by leucocytes, chondrocytes (10), and cells of the synovial membrane (20).

Our previous data (21), which indicated greater involvement of HEX than of cathepsin D in the degradation of joint tissues, suggests the importance of HEX in the rheumatic process and the necessity for research and development of suitable HEX inhibitors which may be injected into the knee joint cavity in the pharmacological treatment of inflammatory diseases of the joints (22).

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