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Assessment of neutrophil elastase plasma levels in children with intermediate uveitis

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Abstract

Intermediate uveitis is a chronic disorder of unclear etiology that affects mainly children and young adults. It can be the first symptom of an autoimmune process and lead to serious disturbances of vision and blindness. Free elastase, strong proteinase released by activated neutrophils plays an important role in the innate immune system and in the pathogenesis of various diseases. As pointed out by many experimental and clinical study elastase in complex with α_1-proteinase inhibitor (NE-α_1PI) may serve as a sensitive indicator of neutrophil activation and inflammation. The aim of the study was to answer the question - is there for the activation of neutrophils in intermediate uveitis?

The research was conducted in 47 patients (21 girls and 26 boys, aged between 4.5-19 years, mean ± SD: 11.77 ±3.69) suffering from idiopathic intermediate uveitis of the stable phase of the disease. The control group consisted of 60 healthy subjects (36 girls, 24 boys, aged 3-18 years, mean ± SD: 10.5 ± 4.46). The concentration of NE-α_1PI was determined in plasma by ELISA.

Elastase concentration (mean ± SD: 174.94 ±183.75 ng/ml, median: 112.76 ng/ml) was significantly (p = 0.000000) elevated compared with controls (mean ± SD: 58.64 ±19.56 ng/ml, median: 56.40 ng/ml).

The results obtained suggest that in children with idiopathic intermediate uveitis comes to activation of circulating neutrophils and elevated levels of neutrophil elastase complexed with α_1-proteinase inhibitor.

Key words: *neutrophil elastase, intermediate uveitis.*

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Introduction

Intermediate uveitis (IU) represents approximately 20% of all forms of uveitis in the pediatric population. The majority of cases is classified as idiopathic. This disorder mostly affects young people, the clinical course is long, may be the first manifestation of systemic disease and can lead to serious disturbances of vision and blindness [1-3].

Studies show that in addition to lymphocytes and macrophages, neutrophils also participate in an ongoing ocular tissue inflammation [4-7]. The increased number of neutrophils, their overstimulation and extended survival, e.g. as a result of delaying apoptosis factors such as the actin-binding protein coronin-1A [8], may escape out of control of the body and cause prolonged inflammation. Hyperactive neutrophils release free radicals and proteolytic enzymes, including neutrophil elastase (NE) into the extracellular environment, leading to degradation of surrounding tissue [9].

Elastase belongs to the most potent proteolytic enzymes. It plays an important physiological function in innate immune system by killing intracellular pathogens [10] as well as taking part in the killing process called Neutrophil Extracellular Traps (NETs) [11]. After being released from neutrophils, elastase should be quickly inactivated by natural inhibitors in systemic fluids [12]. Elevated concentration of free NE, is particularly important in several respects. Firstly, when it is released from over activated neutrophils or from phagocytes after their apoptosis, and when it is unbounded with natural inhibitors, NE demonstrates potent protease activity can destroy extracellular matrix components (EMC), basement membranes [13], and promotes microvascular injury [14]. Through the ability to break down the EMC, neutrophil elastase allows pro- and anti-

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inflammatory mediators and cells to pass through the wall of blood vessels to sites of inflammation. The ability of NE to inactivate the complement components C3 and C5a, immunoglobulin, proteinase inhibitors, clotting factors and adhesion molecules have a direct impact on the course of inflammatory reactions [9]. Secondly, the concentration of neutrophil elastase forming a complex with α_1-proteinase inhibitor (NE-α_1PI) correlates with the released free NE elastase and can be used as a measure for the activity of neutrophils during an inflammatory response in different diseases [15-18].

To our knowledge, there are very few reports on neutrophil activation and role of NE in inducing uvea damage and development of a chronic inflammation of ocular tissues in patients suffering from uveitis [7, 19]. The aim of the study was to evaluate NE-α_1PI in the blood of patients with idiopathic intermediate uveitis.

Material and methods

Subjects

Table 1 presents the characteristics of the study groups. The study was carried out on a group of 47 children (84 affected eyes) aged from 4.5 to 19 years, of both sexes suffering from idiopathic intermediate uveitis of the stable phase of the disease. The patients were treated at the Department and Clinic of Ophthalmology, Medical University, Wroclaw, Poland. Diagnosis of IU was based on clinical classification of uveitis according to the International Uveitis Study Group [20]. The uveitis occurred in both eyes in 37 (78.72%) our patients, which is comparable with literature data [3]. All patients included to the study had a complete ophthalmological examination, were clinically stable and none of them had active disease or acute infection (erythrocyte sedimentation rate, C-reactive protein and total white blood cell count were within the normal range). All were without clinical and laboratory symptoms of autoimmune diseases. None of the patients had primary immunodeficiency. In order to detect causes of intermediate uveitis serologic testing for Lyme disease, toxoplasmosis, toxocarosis, and screening chest X-rays were performed. At the time of testing, none of our patients did not receive topical or systemic anti-inflammatory and/or immunosuppressive therapy.

Controls included 60 healthy children (Table 1), both sexes, without signs any ocular disorders and chronic systemic diseases and without treatment in anamnesis.

The study was approved by the Research Ethics Committee at the Medical University of Wroclaw, and informed consent was obtained from all patients and controls. The children were enrolled for the study with parental agreement.

Blood samples

The material for investigations included venous EDTA-treated blood samples which were centrifuged (3000 rpm, 10 min) within two hours of collection. The remaining plasma was immediately divided into aliquots and stored at –80°C until assayed.

Neutrophil elastase measurement

The ELISA method was used to determine the concentration of circulating in plasma human neutrophil-elastase (ng/ml), in complex with α_1-proteinase inhibitor (NE-α_1PI), using reagents manufactured by IBL International GmbH, Hamburg, Germany. The analyses were performed according to the manufacturers' recommendations. The limits of detection for NE-α_1PI was 1.98 ng/ml.

Statistical analysis

Normality of the distribution of data was assessed with the Kolmogorov-Smirnov test. The obtained results were subjected to statistical analysis using the Student's *t*-test to compare normally distributed unpaired variables and the nonparametric Mann-Whitney U test for non-normal distribution. The level of statistical significance was assumed to be $p < 0.05$. Statistical analyses were performed using StatSoft software (STATISTICA 8).

Results

The results of NE-α_1PI level are given in Table 2 and Fig. 1. All IU patients were divided into two groups depending on the presence of bilateral ($n = 37$, 78.72%) or unilateral ($n = 10$, 21.28%) changes in the eyes. The plasma level of NE-α_1PI showed a significant increase across the three groups (all IU, bilateral IU and unilateral IU) compared with the control group ($p = 0.000000$, $p = 0.000000$, $p = 0.000001$, respectively). NE-α_1PI concentrations above

Table 1. Clinical characteristic of the patients with intermediate uveitis (IU) and healthy controls

Group	All IU patients	Patients with bilateral IU	Patients with unilateral IU	Control group
number (*n*)	47	37	10	60
sex: female (%)	21 (45%)	14 (38%)	7 (70%)	36 (60%)
male (%)	26 (55%)	23 (62%)	3 (30%)	24 (40%)
age (range; mean ± SD, years)	4.5-19; 11.77 ±3.69	4.5-19; 11.12 ±3.54	10-19; 14.5 ±3.34	3-18; 10.5 ±4.46

Table 2. Plasma concentrations of NE-α_1PI in the intermediate uveitis (IU) patients and healthy controls

NE-α_1PI (ng/ml)	All IU patients (n = 47)	Patients with bilateral IU (n = 37)	Patients with unilateral IU (n = 10)	Control group (n = 60)
mean ± SD	174.94 ±183.75	187.56 ±200.61	128.26 ±92.30	58.64 ±19.56
median	112.76	112.76	112.32	56.40
range (min-max)	30.88-877.70	42.96-877.70	30.88-344.60	28.70-98.60
interquartile range (25-75%)	74.70-178.70	75.93-179.50	71.18-163.20	42.8-75.20

the normal range (58.64 ±39.12 ng/ml, mean ± SD) were observed in 26/47 (55%) all IU patients, 21/37 (57%) with bilateral and in 5/10 (50%) with unilateral disease. We found no significant difference in the concentration of elastase between bilateral IU and unilateral IU patients.

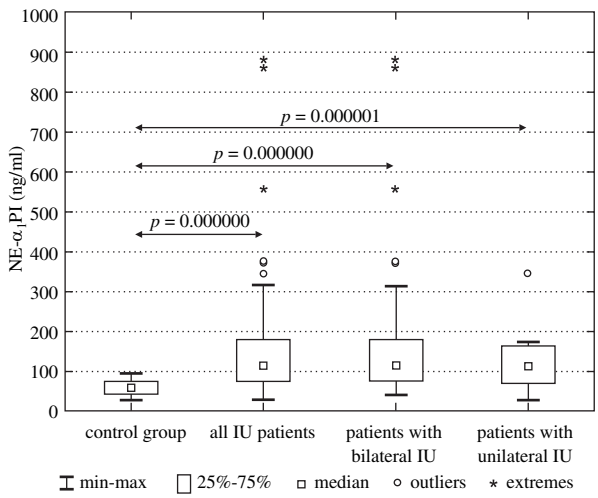

Fig 1. Plasma NE-α_1PI levels in the patients with intermediate uveitis (IU) and healthy controls

Discussion

The importance of neutrophils in the pathogenesis of many diseases including the ocular tissue inflammation remains a subject of intensive research. The results of this study show that circulating in plasma neutrophil elastase is present in elevated concentrations in patients with idiopathic intermediate uveitis. It is comparable with a previous study by Akyol *et al*. [19] who reported that plasma levels of elastase in patients with uveitis (anterior and intermediate - both taken together) prior to treatment was significantly increased compared to healthy control (mean ± SD 305 ±177 µg/l, median 281 µg/l, min 96 µg/l, max 897 µg/l; mean ± SD 43 ±26 µg/l, median 46 µg/l, min 8 µg/l, max 91 µg/l, respectively). They also showed a significant correlation between the effectiveness of treatment and the clinical ocular findings compared to the dynamics of changes in concentration elastase. If no improvement in the applied topical therapy or relapse of the changes in the eyes of elastase levels were elevated. The fact, that our IU patients did not show any signs of acute infection may suggest that chronic- or micro-inflammation connected with overstimulation of neutrophils were presented in these cases.

Ongoing in the uvea, a highly vascularized tissue and rich in various inflammatory cells and mediators, of chronic inflammation (despite the image of remission in the clinical trial) may lead to the development of a number of intraocular complications [3]. Chronic intermediate uveitis may show cellular infiltration in the vitreous, vitreous fibrosis, vitreoretinal fibrosis, pars plana exudates ("snowbanking") [21].

Research has shown that elastase is found in the tissues of the eyes, for example, subretinal [22] or vitreous fluid samples [7]. The question is – what does it mean the presence of elastase in the tissues of the eye. It is not clear why there is the over-stimulation of neutrophils and the presence of an excess of free elastase in patients with IU, especially in the absence of clinical signs of exacerbation. It may partly result from the natural desire of the body to remove harmful substances or fragments of pathologically altered tissue. Studies in experimental animal models indicate that the accumulation of neutrophils in uveitis may occur due to induction of endotoxin [5] and the ability of ocular tissue for the production of the chemokine CINC (cytokine-induced neutrophil chemoattractant) [23]. DeBoer and co-authors [7] point to the important role of interleukin-8, the major chemotactic factor that affects the migration of neutrophils to the site of inflammation in patients with uveitis. NE in complex with α_1PI may be also a neutrophil chemoattractant [24]. On the other hand, experimental data on mice suggest that when there is a lack of NE, cells emigration is impaired and retention of neutrophils occurs. This disorder leads to neutrophil-related endothelial cell injury and increase vasculature permeability [25, 26].

Until recently, mainly pointed to the destructive action of free elastase. However, the results of a number of papers indicate that in some cases this may be beneficial for the body. In the case of long-term inflammatory elastase may have a double, opposing action [13, 27, 28]. On the one hand, it breaks down and destroys a number of components of connective tissue matrix (e.g. elastin, collagen, fibro-

nectin, proteoglycans, fibrin), that is can also cause destruction of ocular tissue [9]. At the same time it can be prevented fibrosis, one of the major complications of chronic uveitis [21]. Owen *et al*. [29] suggest that even in the presence of proteinase inhibitors, NE can degrade extracellular matrix as a result of binding to the cell surface and preservation of the catalytic activity. It is very interesting and important that NE by itself may degrade α_1PI, so that the inhibitor cannot protect the surrounding tissues from enzyme-mediated destruction [30]. Cleavage of α_1PI also generates peptide fragments that have neutrophil chemotactic activity. But on the other hand, NE is also paradoxically involved in excessive EMC deposition and fibrotic process, which is observed for example in the pulmonary fibrosis [15]. Although the role of elastase for these pathologic states is still unclear, some data suggest that one of the potential way of this remodeling is the availability of this proteinase to releasing latent tumor necrosis factor-β (TGF-β) in the ECM and increasing the generation of the active TGF-β resulting in fibrogenesis [31].

Patients with noninfectious and idiopathic uveitis during the development of the disease have an increased tendency to autoimmunity against the eye tissue breakdown products that become autoantigens, such as uveal melanin, retinal arrestin or interphotoreceptor retinoid-binding protein (IRBP) [32]. A study done by Decamps and colleagues [33] suggest that neutrophil elastase degrades the IRBP, a dominant autoantigen in autoimmune uveitis, into an immunoreactive peptide [34] which can initiate or exacerbate the immune response against IRBP and generate autoimmunity.

Full answer to the question what role does neutrophil elastase in the pathogenesis of uveitis requires further study. This takes on special importance when the sick children and young adults [1-3]. Patients with elevated levels of elastase should be subject to detailed and multispecialist research in order to clarify the reasons for the increased activity of neutrophils. With the advancement of knowledge about the participation of neutrophils in inflammatory processes, it is proposed to search for new treatment options such as substances that inhibit the secretion of neutrophil chemotactic factors and inhibitors block neutrophil elastase.

Conclusions

Increased levels of neutrophil elastase demonstrated in this study indicates that this powerful proteolytic enzyme may be involved in the eyes inflammation. Due to the fact that neutrophil elastase is released during neutrophil degranulation of their azurophilc granules, it may be regarded as an indicator of activation of these cells in patients suffering from intermediate uveitis.

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