

The urokinase system in patients with intermittent and persistent allergic rhinitis

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Local and systemic changes in hemostasis are associated with allergic diseases. Apart from their well documented role in regulation of plasminogen activation, components of the urokinase system may be involved in modulation of cellular activities during immune inflammatory responses. So far, little has been known about the function of the system in allergic inflammation. In the present study, we assessed circulating levels of the urokinase system components such as urokinase-type plasminogen activator (uPA), soluble form of uPA receptor (CD87), and the inhibitor – plasminogen activator inhibitor type 1. The study comprised of patients suffering from allergic rhinitis, however, without any asthmatic symptoms. Plasma levels of uPA and soluble form of uPA receptor antigens, and plasminogen activator inhibitor type 1 activity were measured in 17 patients with grass pollens-induced intermittent allergic rhinitis and 15 patients with persistent allergic rhinitis due to house dust mite allergy, as well as in 20 sex-matched and age-matched healthy nonatopic participants. We did not observe any statistically significant differences in the levels of the urokinase system components between patients with intermittent allergic rhinitis and persistent allergic rhinitis,

and the controls. The circulating levels of uPA, its soluble receptor, and its inhibitor did not differ between allergic rhinitis patients and healthy participants, therefore it seems that the systemic release and activity of the urokinase system molecules may be not significantly changed in the course of nasal allergic inflammation induced by periodic or continuous exposure to a natural allergen. *Blood Coagulation and Fibrinolysis* 19:685–688 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Urokinase-type plasminogen activator (uPA), its cell surface receptor (uPAR, CD87), and its inhibitor, plasminogen activator inhibitor type 1 (PAI-1) form a system, named the urokinase or the uPA/uPAR/PAI-1 system. Apart from its well recognized role in fibrinolysis, the system is involved in many other biological processes. Components of the system are expressed by numerous different cell types, and they are important regulators and mediators of immune inflammatory processes, partly because of their participation in mechanisms mediating recruitment and migration of cells as well as inflammatory mediators and immune stimuli affecting their synthesis [1,2]. The cells playing the key role in allergic inflammation are also capable of expressing functionally either active uPA or its receptor or both, including dendritic cells [3], eosinophils [4,5], and mast cells [6]. In addition, uPAR expression on eosinophils from normal and asthmatic patients was increased by IL-5 [5]. Interestingly, experimental studies demonstrated that the urokinase system might modulate Th1 and Th2 cell response, suggesting significance of the system in diseases in which

such imbalanced activity of the lymphocytes may play an essential role [7,8]. It is well known that local and systemic changes in hemostasis are associated with inflammatory processes. However, a few published data illustrate the circulating levels of the fibrinolytic system components in patients with allergic disease. So far, some changes in the systemic activity of fibrinolytic system have been documented in allergic diseases such as bronchial asthma [9], atopic eczema/dermatitis syndrome (AEDS) [10], and keratoconjunctivitis [11]. However, to our knowledge there are no available data on the behavior of the fibrinolytic system in the systemic circulation of patients with nasal allergic inflammation symptoms. Sejima *et al.* [12] suggested interestingly enough that metabolism and expression of fibrinolytic components at the site of allergic inflammation, such as nasal mucosa, are altered under the influence of cytokines released from inflammatory and immune cells. In the present study we assessed plasma levels of uPA, soluble uPAR, and PAI-1 activity in patients with intermittent and persistent allergic rhinitis. The objective was to determine more precisely the fibrinolytic profile of

patients suffering from allergic diseases. A secondary aim was an attempt at defining whether various clinical forms of allergy might affect the function of the systemic fibrinolytic system in a different manner.

Material and methods

Thirty-two nonsmoking patients with moderate-to-severe symptoms of allergic rhinitis and without concomitant allergy were enrolled in the study. They were divided into two groups: 17 patients with grass pollens-induced intermittent allergic rhinitis (IAR group) and 15 patients with persistent allergic rhinitis due to house dust mite (HDM) allergy (PAR group). Of the 17 symptomatic patients suffering from IAR, seven were women and 10 men; the median age was 24 years (range: 18–29 years). They showed positive skin prick test to grass pollen allergens and no sensitization to other allergens tested, and were examined during natural allergen exposure at the peak of the allergy season. The median value of total immunoglobulin E (IgE) was 278 kU/ml (range: 164–480).

Of the 15 symptomatic patients suffering from PAR, five were women and 10 men; the median age was 25 years (range: 19–35 years). They showed positive skin tests to HDM (either *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae* or both) extract and negative response to all other common aeroallergens or sensitization to other aeroallergens was clinically insignificant. The median value of IgE total was 220 kU/ml (range: 177–432).

Topical nasal medication was withdrawn at least 2 weeks prior to the study. Moreover, none of the examined patients had taken oral antihistamines or leukotriene antagonists within 2 weeks and oral corticosteroids within 8 weeks before the study. None of the patients had received immunotherapy, suffered from diseases, which would affect fibrinolytic activity, or were receiving drugs, which could have affected the function of the fibrinolytic system.

The control group consisted of 20 healthy volunteers, showing no signs of atopy, seven women and 13 men, the median age was 25.5 years (range: 20–32 years). All the participants were nonsmokers and proved normal in physical examination.

The Ethics Committee of the Medical University of Silesia approved this study and written, informed consent was obtained from all the participating subjects.

Blood collection and plasma preparation

The blood was obtained in the morning (0700–0800 h; in the fasting state at least 12 h) after 60-min rest with slight or no stasis from the antecubital vein directly into plastic tubes with anticoagulants (CTAD), and then placed in ice water. The first 2–3 ml of blood were

discarded. The plasma for analyses was separated by centrifugation during 20 min at 2000 *g* and 4°C. All plasma samples were immediately frozen and stored at –30°C until analysis.

Assay of urokinase-type plasminogen activator antigen

Measurement of u-PA antigen in plasma was carried out using enzyme-linked immunosorbent assay (ELISA) kit following the manufacturers' recommendations (Imubind ELISA kits; American Diagnostica Inc., Greenwich, Connecticut, USA).

Soluble urokinase-type plasminogen receptor assay

Soluble uPAR in plasma was measured by a quantitative ELISA kit (R&D, Minneapolis, USA) according to the manufacturers' instructions.

Analysis of plasminogen activator inhibitor type-1 activity

PAI-1 activity in plasma was determined using commercial reagents according to the manufacturers' protocol (Chromolize; Biopool, Umeå, Sweden).

In-vitro immunoglobulin E tests

Serum total IgE was determined using ELISA kit (Allergopharma, Reinbek, Germany), as per the manufacturers' instructions.

Skin prick tests

Allergic status was evaluated by skin prick testing with a panel of common inhalant allergens (Allergopharma). Saline and histamine solutions were used as negative and positive controls, respectively. A wheal of diameter greater by at least 3 mm than the negative control was accepted as a positive response.

Statistical analysis

Data are presented as median with quartiles. Kruskal–Wallis variance analysis was used for screening differences between the groups. Correlation coefficient was obtained by Spearman test. *P*-values below 0.05 were considered significant.

Results

The median and range values of u-PA antigen, suPAR antigen, and PAI-1 activity in plasma from IAR and PAR patients, as well as healthy participants are shown in Table 1. For the entire parameters analyzed no significant differences were observed among the three groups. No significant correlation occurred between serum total IgE values and plasma levels of u-PA, suPAR, and PAI-1 in the patient groups.

Discussion

Allergic diseases such as asthma, rhinitis, and AEDS are inflammatory disorders characterized by local changes at the site of allergic processes. Moreover, the local reactions

Table 1 Plasma levels of components of the urokinase system in patients with intermittent and persistent allergic rhinitis and healthy participants

Analysed parameters (unit)	Control group (n = 20)	IAR group (n = 17)	PAR group (n = 15)
u-PA antigen (ng/ml)	0.38 0.2–0.62	0.44 0.11–0.66	0.5 0.11–0.93
suPAR antigen (ng/ml)	1.47 1.32–1.71	1.65 1.39–2.27	1.52 1.31–1.94
PAI-1 activity (IU/ml)	6.25 4.85–8.8	6.4 5.1–9.1	5.1 4.4–6.0

Values are expressed as median with quartiles. IAR, intermittent allergic rhinitis; PAR, persistent allergic rhinitis; u-PA, urokinase-type plasminogen activator.

are capable of inducing systemic inflammatory and immune response [13–15]. It has been well known that the inflammatory state may alter the performance of the hemostatic system remembering that both local and systemic activation of the coagulation and fibrinolysis system are integral parts of different inflammatory processes. On the one hand, the inflammatory mediators may modulate the balance between procoagulant and anticoagulant activities. On the other hand, however, many components of the hemostatic system have pro-inflammatory or anti-inflammatory properties and may affect inflammatory activity [2,16,17]. In this context, it seems interesting to investigate the behavior and significance of the mediators of the hemostatic system upon various inflammatory processes. Some changes in the blood coagulation parameters, bleeding time or platelet activity have been documented in patients suffering from atopic diseases (for review [18]). Yet only a few studies raised the question whether inflammatory processes due to allergic reactions may alter the functional status of the fibrinolytic system in the peripheral blood and whether the system plays any role in allergic processes. Increased cutaneous and plasma activity of the fibrinolytic system was observed in the acute phase of AEDS, suggesting a role for the system in amplification of the inflammatory phenomenon in the disease [10]. The evidence obtained from the animal model of allergic airway hyperactivity suggests that disordered coagulation and fibrinolysis may contribute to the pathogenesis of asthma [19]. Moreover, Banach-Wawrzęńczyk *et al.* [9] observed increased levels of fibrinolytic markers in the systemic circulation of symptomatic patients with newly recognized asthma, suggesting increased activity of the fibrinolysis system in asthmatic patients. We observed earlier that plasma levels of uPA and suPAR did not differ significantly in patients with AEDS, as compared with healthy participants [20]. In the present study, we have compared the fibrinolytic profile of the patients with allergic rhinitis sensitized to HDM, thus undergoing continuous natural exposure to these aeroallergens, with the patients suffering from intermittent allergic rhinitis, sensitized to grass pollen allergens, who, in our region, are exposed to such pollens during few months of the year. We did not show any significant difference in plasma levels of uPA and suPAR antigens, and PAI-1 activity between patients with intermittent allergic rhinitis, persistent allergic rhinitis, and healthy

nonatopic participants. It seems, therefore, that in the course of nasal allergic inflammation, any significant alterations in the activity of the urokinase system, as measured by the circulated components of the system, are not induced in the systemic circulation of allergic rhinitis patients. Some changes may, however, occur locally at the site of allergic inflammation in the nasal mucosa and discharge. For example, it has been demonstrated that u-PA was activated significantly in allergic rhinitis, suggesting that such activity may help the passage of large amounts of rhinorrhea by reducing its viscosity [12]. In addition, it seems that local imbalance of the urokinase system may be involved in the pathogenesis of severe form of allergic conjunctivitis such as vernal keratoconjunctivitis [11]. On the contrary, the lack of changes in plasma levels of uPA and PAI-1 were reported in patients when compared with healthy participants; in such patients only plasma levels of tissue-type plasminogen activator and plasminogen activity were significantly lower than in the controls. Yet, all the values remained within the normal range [11]. Also, no statistical differences in PAI-1 activity in the blood were observed between patients with newly diagnosed asthma and the healthy participants [9]. However, during an asthma attack, PAI-1 plasma level was significantly higher as compared with the controls [21]. Plasma level of uPA in patients with asthma was significantly lower when compared with healthy participants [9]. Taken together, it seems that some alterations in the function of the fibrinolytic system observed in patients with allergic inflammation are not a general feature of allergic diseases and are not similar throughout different clinical manifestation of allergy.

Conclusion

The present study provided the first report on behavior of components of the urokinase system in the systemic circulation of patients with allergic rhinitis induced by seasonal and continuous natural allergen exposure. As the circulating levels of uPA, its soluble receptor, and its inhibitor did not differ significantly between patients with intermittent and persistent allergic rhinitis and healthy participants, it seems that the systemic release or activity of such molecules incorporated in the urokinase system may not change significantly during immune inflammatory processes associated with allergic rhinitis. Moreover, the present study along with the earlier contributions indicates

that the distinct clinical forms of allergic inflammatory processes may affect systemic fibrinolysis in a different manner.

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References

- Blasi F. uPA, uPAR, PAI-1: key intersection of proteolytic adhesive and chemotactic highways? *Immunol Today* 1997; **18**:415–417.
- Horrevoets AJG. Plasminogen activator inhibitor 1 (PAI-1): in vitro activities and clinical relevance. *Br J Haematol* 2004; **125**:12–23.
- Ferrero E, Vettoretto K, Bondanza A, Villa A, Resnati M, Poggi A, Zocchi MR. uPA/uPAR system is active in immature dendritic cells derived from CD14+ CD34+ precursors and is down-regulated upon maturation. *J Immunol* 2000; **164**:712–718.
- Mabilat-Pragnon C, Janin A, Michel L, Thomaidis A, Legrand Y, Soria C, Lu H. Urokinase localization and activity in isolated eosinophils. *Thromb Res* 1997; **88**:373–379.
- Guilbert M, Ferland C, Bossé, Flamand N, Lavigne S, Lavolette M. 5-Oxo-6,8,11,14-eicosatetraenoic acid induces important eosinophil transmigration through basement membrane components: comparison of normal and asthmatic eosinophils. *Am J Respir Cell Mol Biol* 1999; **21**:97–104.
- Sillaber C, Baghestanian M, Hofbauer R, Virgolini I, Bankl HC, Füreder W, et al. Molecular and functional characterization of the urokinase receptor on human mast cells. *J Biol Chem* 1997; **272**:7824–7832.
- Gyetko MR, Sud S, Chensue SW. Urokinase-deficient mice fail to generate a type 2 immune response following Schistosoma antigen challenge. *Infect Immun* 2004; **72**:461–467.
- Gyetko MR, Sud S, Sonstein J, Polak T, Sud A, Curtis JL. Antigen-derived lymphocyte recruitment to the lung is diminished in the absence of urokinase-type plasminogen activator (uPA) receptor, but is independent of uPA. *J Immunol* 2001; **167**:5539–5542.
- Banach-Wawrzęczyk E, Dziedziczko A, Rość D. Fibrinolysis system in patients with bronchial asthma. *Med Sci Monit* 2000; **6**:103–107.
- Lotti T, Battini ML, Brunetti L, Fabbri P, Panconesi E. Plasminogen activators and antiplasmin activity in atopic dermatitis. *Int J Dermatol* 1989; **28**:457–459.
- Leonardi A, Brun P, Sartori MT, Cortivo R, Dedominicis C, Saggiorato G, et al. Urokinase plasminogen activator, uPA receptor, and its inhibitor in vernal keratoconjunctivitis. *Invest Ophthalmol Vis Sci* 2005; **46**:1364–1370.
- Sejima T, Madoiwa S, Mimuro J, Sugo T, Ishida T, Ichimura K, Sakata Y. Expression profiles of fibrinolytic components in nasal mucosa. *Histochem Cell Biol* 2004; **122**:61–73.
- Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001; **108** (Suppl 5):S147–S334.
- Borish L. Allergic rhinitis: systemic inflammation and implications for management. *J Allergy Clin Immunol* 2003; **112**:1021–1031.
- Jousilahti P, Salomaa V, Hakala K, Rasi V, Vahtera E, Palosuo T. The association of sensitive systemic inflammation markers with bronchial asthma. *Ann Allergy Asthma Immunol* 2002; **89**:381–385.
- Emeis JJ, Klufft C. PAF-acether-induced release of tissue-type plasminogen activator from vessel walls. *Blood* 1985; **66**:86–91.
- Cicala C, Cirino G. Linkage between inflammation and coagulation: an update on the molecular basis of the crosstalk. *Life Sci* 1998; **62**:1817–1824.
- Kasperska-Zajac A, Rogala B. Platelet activation during allergic inflammation. *Inflammation* 2006; **30**:161–166.
- Matthay MA, Clements JA. Coagulation-dependent mechanisms and asthma. *J Clin Invest* 2004; **114**:20–23.
- Kasperska-Zajac A, Rogala B. Circulating levels of urokinase-type plasminogen activator (uPA) and its soluble receptor (suPAR) in patients with atopic eczema/dermatitis syndrome. *Inflammation* 2005; **29**:90–93.
- Tutluoglu B, Gurel CB, Ozdas SB, Musellim B, Erturan S, Anakkaya AN, et al. Platelet function and fibrinolytic activity in patients with bronchial asthma. *Clin Appl Thromb Hemost* 2005; **11**:77–81.