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Plasminogen activators in human nasal polyps and mucosa*

Knud Larsen[1], Moniek P.M. de Maat[2], Jørgen Jespersen[2]

[1] Ear, Nose and Throat Clinic, Ribe County Hospital, South Jutland University Centre, Esbjerg, Denmark
[2] Department of Clinical Biochemistry, Institute for Thrombosis Research, South Jutland University Centre, Esbjerg, Denmark

SUMMARY

Fibrinolysis is one of the processes that are involved in inflammation. In this study we have investigated if it is also involved in bilateral nasal polyposis, a disease with an inflammatory component. Fibrinolytic activity in the nasal mucosa and nasal polyps has been studied in 10 patients with bilateral nasal polyposis. Tissue-type plasminogen activator (t-PA) and urokinase-like plasminogen activator (u-PA) activities and antigen levels have been determined in polyp tissue and control nasal mucosa. t-PA activity is higher in nasal mucosa (median: 4.26 IU/mg) as compared with nasal polyps (median: 0.65 IU/mg; p=0.03); u-PA activity is slightly lower in nasal mucosa (median: 0.040 IU/mg) as compared to polyps (median: 0.065 IU/mg; not significant). The percentage of u-PA to t-PA is 7.9% in nasal mucosa and 22.8% in nasal polyps (p<0.01). The shift towards a higher u-PA/t-PA ratio in nasal polyps suggests an inflammatory process. Plasma levels of C-reactive protein are all within normal limits, which may suggest that PA activity is restricted to a local inflammatory reaction in the airway mucosa. The higher u-PA/t-PA ratio in nasal polyps and the higher levels of u-PA, when compared with the findings in other organs affected by inflammation, indicate that u-PA plays a part in the inflammatory events resulting in nasal polyps.

Key words: tissue-type plasminogen activator, urokinase-like plasminogen activator, nasal polyposis, fibrinolysis, inflammation

INTRODUCTION

Knowledge about the pathogenesis of bilateral nasal polyposis is still insufficient, but it is known that polyps are histologically characterized by an eosinophilic inflammation (Mygind, 1990). Fibrinolysis is one of the processes that are involved in inflammation (Astrup, 1968), both in animal models and in pathological states in man, as has been highlighted in a review by Danø et al. (1985).

Plasminogen activators are glycoproteins that convert the inactive zymogen plasminogen into the active enzyme plasmin. Two types of plasminogen activators are known, tissue-type (t-PA) and urokinase-like (u-PA; Danø et al., 1985). These two plasminogen activators are the products of two separate genes. t-PA binds with a high specificity to fibrin, the insoluble protein polymer in thrombi, and is therefore considered to be the major plasminogen activator that participates in the lysis of blood clots (Astrup, 1978). u-PA does not bind to fibrin but to a specific u-PA receptor, and has been shown to possess proteolytic activity, mitogenic and growth factor-like properties and plays a role in tissue remodelling (Danø et al., 1985).

In adenomatous colon polyps and colitis ulcerosa a shift towards a relatively higher u-PA activity over t-PA activity has been observed (De Bruin et al., 1987; De Jong et al., 1989), indicating the role for u-PA in the inflammatory process. Whether a similar relation can be found between t-PA and u-PA activity in the nasal mucosa and in nasal polyposis has not yet been studied. However, it is known that general fibrinolytic activity is higher in sinus mucosa with chronic sinusitis than in nasal mucosa and polyps (Sasaki et al., 1959). More specific analyses have shown that at least t-PA activity is present in sinus mucosa with chronic infection (Kosugi et al., 1982; Yamashiro et al., 1993) and that both t-PA and u-PA activity are present in antrochoanal polyps (Yamashiro et al., 1992). The antrochoanal polyp represents a clinically separate entity associated with chronic antral sinus inflammation, most often unilateral. Nasal polyposis originating from the ethmoidal region makes up a another clinical entity, most often with bilateral presentation. Meanwhile, in both entities, eosinophilic inflammation can be present (Cook et al., 1993).

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The aim of the present study was to evaluate the specific u-PA and t-PA activities in nasal polyps and nasal mucosa and to study the relation between these activators in the two tissues.

MATERIAL AND METHODS

Patients and tissue samples

Ten consecutive patients that were scheduled for endoscopic polypectomy were enrolled in this study. Nine were males and one female, the median age was 54 years (range: 43-66 years). Five patients had asthma, one patient had an allergy. None of the patients had acetylsalicylic acid intolerance, none had treatment for sinusitis or treatment with topical steroid within one month, and none had systemic steroid within 3 months before polypectomy.

The polyps were collected between 08.00 a.m. and 10.00 a.m., immediately washed in 154 mM NaCl to remove blood and stored frozen at –80°C until extraction. From each patient, also a biopsy of normal nasal mucosa was taken from the inferior turbinate, just below the anterior end of the medial turbinate on the same side as the polyps.

The median duration of recognized nasal polyposis was 5.5 years (range: 0-13 years), two patients presented with their first episode of polyps. Approval for the study was given by the Ethical Committee of the County of Ribe, Denmark.

Quantification and classification of plasminogen activator activity

Extracts of the polyp and normal nasal mucosa were prepared by homogenizing 5-60 mg of wet tissue samples in 1,000 µl thiocyanate buffer (1 M KSCN, 0.25% (w/v) gelatin; pH 7.75) followed by centrifugation at 2,900*g* during 10 min at room temperature (Astrup et al., 1971).

Total fibrinolytic activity was determined using the highly-standardized fibrin plate method (Astrup and Mullertz, 1952; Brakman, 1967; Jespersen and Astrup, 1983). Briefly, a fixed volume of tissue extract was placed in duplicate on plasminogen-rich fibrin plates and incubated for 17 hours at 37°C. The diameter of the lysis zone was then measured and used to calculate the fibrinolytic activity. Single-chain t-PA (Biopool, Umeå, Sweden) and low-molecular u-PA (American Diagnostic, Greenwich, Connecticut, USA) were used as reference material. t-PA and u-PA activities were determined by adding specific antibodies against t-PA (rabbit-IgG against human t-PA; Organon, The Netherlands) and u-PA (goat-IgG against human u-PA; Organon, The Netherlands) to parallel incubations and calculating the amount of inhibition. Residual activity after the addition of both anti-t-PA and anti-u-PA antibodies is given as mm^2.

The linear dilution curves in a double-logarithmic graph of the dextran sulphate fraction (DEF) of pooled plasma and extracted or purified t-PA and u-PA are parallel (Jespersen and Astrup, 1983). This characteristic was used to calculate the concentrations of t-PA activity and u-PA activity in the tissue extracts. The squared diameter (mm^2) of each lysis zone was converted to DEF-units using a dextran sulphate dilution curve. Then the lysis zone from the sample with anti-t-PA was subtracted from that with no antibodies and this activity was ascribed to t-PA. The DEF units were then reconverted to mm^2 which were then transformed to International Units (IU) of t-PA using a t-PA dilution curve. A similar procedure was used to calculate IU of u-PA.

t-PA antigen in the tissue extracts was determined using an enzyme immunoassay or EIA (Imulyse t-PA-kit; Biopool, Umeå, Sweden; cf. Rånby et al., 1986). u-PA antigen in the tissue extracts was determined using an EIA (Tint Elisa u-PA-kit). C-reactive protein (CRP) in serum was measured with a sensitive EIA, which uses rabbit antibodies against human CRP as catching and tagging antibodies (DAKO A/S, Glostrup, Denmark).

Statistical analysis

Non-parametric statistical methods were used, namely the Mann-Whitney test and the Wilcoxon's test. A p-value <0.05 was considered significant.

Table 1. t-PA and u-PA activity and antigen levels in nasal mucosa tissues and nasal polyps.

	mucosa		polyps		
	median	range	median	range	P
t-PA activity (IU/mg)	4.260	0.3-26.38	0.650	0.34-1.99	0.03
u-PA activity (IU/mg)	0.040	0-0.23	0.065	0.01-0.27	NS
% t-PA	93.0	80-100	78.0	63-91	0.01
% u-PA	7.0	0-20	22.0	9-37	0.01
t-PA antigen (ng/mg)	0.325	0-9.86	0.002	0-3.74	NS
u-PA antigen (ng/mg)	<0.1		<0.1		

% t-PA and % u-PA are the percentage t-PA and u-PA of the total fibinolytic activity.

RESULTS

t-PA and u-PA activities in the nasal mucosa and nasal polyps are given in Table 1. t-PA activity is significantly higher in nasal mucosa than in nasal polyps ($p<0.05$); u-PA activity in nasal mucosa appears to be slightly lower than in polyp tissue (not significant). The median percentage t-PA activity of the total PA activity in mucosa is 93% (range: 80-100%) and in polyps 78% (range: 63-91%; $p=0.01$). This suggests that there is a shift towards a relatively higher u-PA activity in the polyps.

Residual fibrinolytic activity was neglectible and was not taken into account. Only seven of the mucosa biopsies and two polyps showed very small amounts of residual activity (not significant). There was no difference in t-PA or u-PA activity levels in the tissue of patients with or without asthma, neither in polyps nor in nasal mucosa. No apparent links could be detected between t-PA and u-PA activity levels in polyps or nasal mucosa and the clinical activity or duration of the polyp disease, size of polyps, degree of eosinophilia and the presence of bacteria on the polyp surface. t-PA antigen levels in the tissues were comparable in mucosa and polyps (Table 1); t-PA antigen levels were below detection limits in four mucosa biopsies and in seven polyps.

u-PA antigen levels in mucosa and polyps were all below the detection limits for the method used. CRP levels were all within the clinical normal limit (10 mg/l) with a median of 1.2 mg/l (range: 0.2-4.5 mg/l), and no difference between patients with or without asthma was observed.

DISCUSSION

t-PA activity in the nasal mucosa was nearly 7 times higher than the activity in the nasal polyps; u-PA activity in polyps was 1.6 times higher than in the nasal mucosa. The percentage u-PA activity of the total PA activity was thus larger in the nasal polyps than in the nasal mucosa. This shift towards a relatively higher u-PA activity in the polyps may be a reflection of the inflammatory process, characteristic for polyps. However, the difference in u-PA/t-PA ratio between nasal mucosa and polyps can to a large extent be explained by the very high t-PA activity levels in the nasal mucosa.

Changes in u-PA/t-PA ratio have also been observed in colon biopsies (De Bruin et al., 1987; De Jong et al., 1989). The contribution of u-PA to the total activity was 6% in normal colon mucosa and 20% in adenomatous colon polyps (De Bruin et al., 1987). In patients with colitis the percentage of u-PA in non-involved mucosa was 29% and this was comparable with a normal control group, while the percentage of u-PA activity in disease-involved mucosa was 45% (De Jong et al., 1989). These findings indicate a shift towards a relatively higher u-PA activity in the tissues in the presence of inflammation.

In our study, we have expressed the fibrinolytic activity relative to the wet weight of the tissue samples. This means that we may have underestimated the levels of fibrinolytic activity in the polyp tissue, since this was more oedematous than the nasal mucosa. However, if we had chosen to express fibrinolytic activity relative to the protein content of the tissue instead, it would not have affected the estimated ratio of u-PA/t-PA in the two tissue types, nor the present results.

In another study (Yamashiro et al., 1992) only t-PA activity was detected in the paranasal mucous membrane affected by chronic sinusitis, a contradictory finding to the statement of a shift towards more t-PA activity in inflammation. Quantitatively, t-PA activity in nasal mucosa in the present study was higher than those observed in colon mucosa in the above-mentioned studies (De Bruin et al., 1987; De Jong et al., 1989). Markedly higher levels of both u-PA and t-PA activity were found in both nasal polyps and nasal mucosa, when compared with the findings in colon mucosa with colitis ulcerosa (De Jong et al., 1989). t-PA levels in tissues from different organs show large variations (Rijken, 1980), but in all cases t-PA levels were less than those observed here.

Although the higher u-PA/t-PA ratio in nasal polyps suggest an inflammatory process, this is not supported by other markers of inflammation, neither by clinical data nor by plasma levels of C-reactive protein. Therefore, our observations might imply that PA activity is restricted to a local inflammatory reaction in the airway mucosa. Since t-PA is secreted by endothelial cells, a possible explanation for the difference between t-PA activity in the nasal mucosa and the polyps could be that the amount of vessels in the two tissues differs or that there is a difference in t-PA production between the different endothelial cells, as has been described by Van Hinsberg (1988).

In summary, very high levels of t-PA were observed in nasal mucosa from the inferior turbinate, and the levels of u-PA activity were comparable in the polyps and the nasal mucosa. Our observations of a higher u-PA/t-PA ratio in nasal polyps, and higher levels of u-PA when compared to the findings in other organs affected by inflammation, indicate that u-PA plays a part in the inflammatory events resulting in nasal polyposis.

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Knud Larsen, MD
ENT Clinic
Jernbanegade 22
DK-6700 Esbjerg
Denmark