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Secretory leukocyte protease inhibitor in normal, allergic and virus induced nasal secretions

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SUMMARY

Secretory leukocyte protease inhibitor (SLPI) and α-1-proteinase inhibitor (α-1-PI), both inhibitors of granulocyte elastase, were studied in nasal secretions from healthy persons and from patients with allergic rhinitis and common cold. SLPI and granulocyte elastase were found in all samples, while α-1-PI was lacking in several. In all three groups SLPI was found in an active form and in excess of granulocyte elastase, which thus was completely inhibited. The results indicate that SLPI is the main inhibitor in nasal secretions and that α-1-PI plays a minor role.

INTRODUCTION

The dominating inhibitors of granulocyte elastase in the respiratory tract are the plasma protease inhibitor α-1-PI and a locally produced, acid stable, low molecular weight inhibitor (Ohlsson and Tegner, 1976). The structure of the low molecular weight inhibitor has been the subject of a number of conflicting reports (Ohlsson et al., 1986). The confusion can be attributed to the fact that degraded protein was used in the structure determination. Recently an inhibitor which cross-reacts with antibodies raised against the low molecular weight inhibitor from bronchial secretions was isolated from parotide juice and the structure determined (Thompson and Ohlsson, 1986). The inhibitor was named secretory leukocyte protease inhibitor (SLPI) and it is established that SLPI is the inhibitor produced in respiratory tract mucosa, genital tract and salivary glands (identical with antileukoprotease, HUSI-I, CUSI-I and BMI) (Schiessler et al., 1978; Ohlsson et al., 1986).

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Granulocyte elastase is present in purulent bronchial secretions where it is the dominating cause of the elastolytic activity (Ohlsson and Tegner, 1975). Granulocyte elastase is released in connection with cellular destruction and phagocytosis and has been shown to produce emphysema in experimental animals (Senior et al., 1977; Ohlsson and Olsson, 1977). The ciliated respiratory mucosa is easily damaged by granulocyte elastase in vitro. SLPI effectively protects against such experimentally induced destruction (Tegner et al., 1979). During purulent rhinitis the nasal mucosa is exposed to massive amounts of granulocyte proteases. It is therefore conceivable that the mucosa is protected by an efficient barrier of protease inhibitors. α-1-PI and acid stable low molecular weight inhibitors have been identified in such secretions earlier (Hochstrasser et al., 1971). The purpose of this study was to investigate the content of the inhibitors α-1-PI and SLPI and of granulocyte elastase in nasal secretions from healthy persons as well as from patients with allergic rhinitis and common cold.

MATERIAL AND METHODS

Healthy persons

Nasal secretions from 23 healthy persons, without a history of allergic rhinitis or of nasal infection the preceding three weeks, were gently collected by suction. The nasal secretions were aspirated into the collection tube and diluted with 0.5 ml Tris-buffer (0.05 M Tris-HCl buffer, pH 7.6).

Patients with allergic rhinitis

Samples of nasal secretions were obtained from 16 patients with birch pollen allergy. Into each nostril 0.1 ml of birch pollen extract was sprayed with a De Vilbis spray No. 15. The challenges were carried out with highly purified birch pollen extracts using increasing concentrations until a convincing positive reaction was elicited consisting of sneezing, nasal blockage and hypersecretion. The patients were then asked to sit for 10 minutes in a bent forward position and to breathe through the mouth. Nasal secretions dripping were collected in a funnel connected to a graded syringe.

Patients with common cold

Six healthy persons were inoculated in the nose by rhinovirus (Gaffey et al., 1987). Rhinovirus type 39 was administered intranasally by drops (0.25 ml per nostril) on two occasions four to five hours apart. After three and four days 5 ml of isotonic saline solution was injected into each nostril of the patients. The head was hyperextended and the nasopharynx was voluntarily held closed. On flexion of the head, the nasal secretion was drained and collected into tubes.

All samples were collected in tubes and frozen at $-20°$ C.

The study was approved by the Ethics Committee of the Medical Faculty, University of Lund, Sweden.

Sample analysis

All samples were analysed for SLPI, α-1-PI, albumin, granulocyte elastase and granulocyte elastase activity. One sample of nasal secretions from each group of patients was subjected to gel-filtration. The fractions were analysed for SLPI, granulocyte elastase and α-1-PI.

Electroimmunoassays were used for quantification of α-1-PI and albumin (Laurell, 1972).

Radioimmunoassays were used for measurements of SLPI and granulocyte elastase (Fryksmark et al., 1981; Ohlsson and Olsson, 1978).

Gel-filtration of nasal secretions was performed on a Sephadex G-75 column (0.9 × 60 cm), equilibrated with 0.05 M Tris-HCl buffer, pH 7.6, containing 0.6 M NaCl and 0.002 M $CaCl_2$.

Special material

Sephadex G-75 was purchased from Pharmacia Fine Chemicals AB, Uppsala, Sweden. Agaros Seakem (batch 50013) was obtained from Marine Colloids Div. FMC Corporation, Rockland, USA and L-Pyroglutamyl-L-prolyl-L-valine-p-nitroanilide (S-2484) was a product from Kabi Diagnostica, Stockholm, Sweden. The birch pollen extract used was Spectralgen® from Pharmacia, Sweden.

Virus

The rhinovirus type 39 (T-39 IF4-13 2-4-83 WI-2) was a kind gift from Dr J.M. Gwaltney Jr and has been used in a previous study (Gaffey et al., 1987). The virus has been found to give mild symptoms of a common cold consisting of rhinorrhoea, nasal congestion and sore throat, lasting for a few days.

RESULTS

1. Healthy persons

Nasal secretions, diluted with Tris-buffer, from 23 healthy persons contained 2.7 to 79 mg/l of SLPI; 0 to 21 mg/l of α-1-PI; 0.197 to 4.12 mg/l of granulocyte elastase and 10 to 855 mg/l of albumin.

2. Allergic rhinitis

Undiluted nasal secretions from 16 patients with allergic rhinitis challenged with birch pollen extracts contained 6.3 to 195 mg/l of SLPI. α-1-PI varied from 0 to 32 mg/l. Granulocyte elastase was found in a concentration from 0.032–2.286 mg/l and albumin from 90–1800 mg/l.

3. Common cold

Nasal secretions, diluted with isotonic saline solution, from six patients exposed to rhinovirus were recovered in 14 samples. One sample was collected from each

patient on day 3 and one sample from four patients and two samples from two patients (one from each nostril) on day 4. Two patients developed a severe rhinovirus cold and four patients got milder symptoms with only nasal congestion and sore throat. Diluted nasal secretions from these patients contained 0.2 to 4.8 mg/l of SLPI; 0 to 2.0 mg/l of α-1-PI; 0.005 to 1.040 mg/l of granulocyte elastase and 2.2 to 43 mg/l of albumin.

4. Ratio of SLPI/albumin and granulocyte elastase/albumin

Due to the different methods in collecting nasal secretions, SLPI and granulocyte elastase were correlated to the albumin content in the different nasal secretions (Table 1).

Table 1. Mean values and differences (p) of the ratio of SLPI and albumin and of granulocyte elastase and albumin in nasal secretions from the different groups of patients.

	n	mean ± SD	groups compared	difference (p)
SLPI/albumin				
healthy persons (HP)	23	0.365 ± 0.368	HP/AR	$p < 0.05$
allergic rhinitis (AR)	16	0.141 ± 0.173	HP/CC	$p < 0.02$
common cold (CC)	14	0.111 ± 0.067	CC/AR	N.S.*
granulocyte elastase/albumin				
healthy persons (HP)	23	0.021 ± 0.014	HP/AR	$p < 0.001$
allergic rhinitis (AR)	16	0.001 ± 0.001	HP/CC	$p < 0.01$
common cold (CC)	14	0.007 ± 0.008	CC/AR	$p < 0.01$

* N.S. = no significance

In nasal secretions from healthy persons the SLPI concentration was higher correlated to the albumin content than in patients with allergic rhinitis ($p < 0.05$) and in patients with common cold ($p < 0.02$). The ratio of SLPI/albumin showed no difference in nasal secretions from patients with allergic rhinitis and patients with common cold.

The ratio of granulocyte elastase/albumin showed that the content of granulocyte elastase in nasal secretions from healthy persons was higher than in nasal secretions from patients with common cold ($p < 0.01$) and allergic rhinitis ($p < 0.001$) (Table 1).

5. Gel-filtration of nasal secretions

In nasal secretions from a healthy person and from a patient with common cold, the dominating part of SLPI was found in a free form and only a small part was

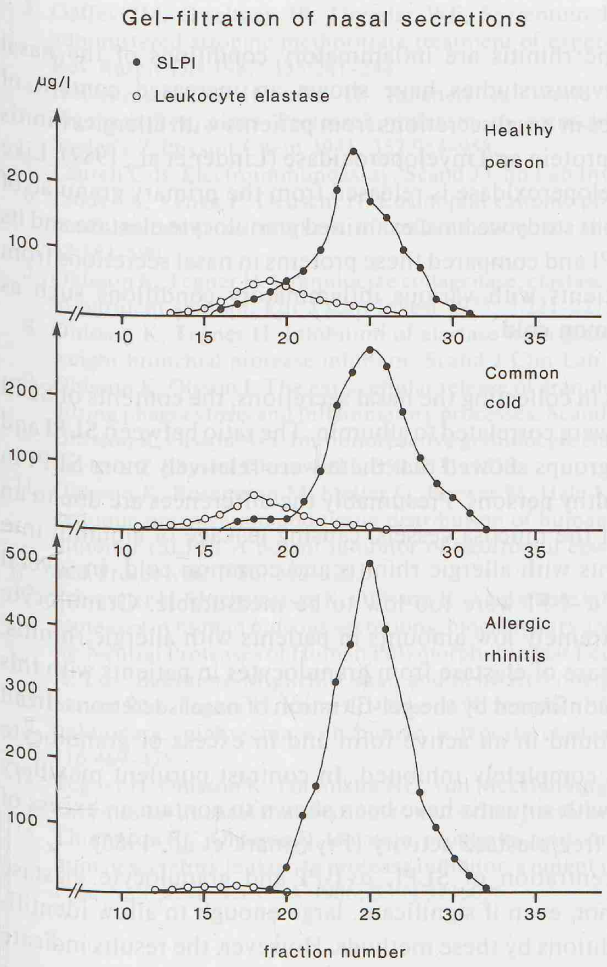

Figure 1. Partition of SLPI and granulocyte elastase in fractions obtained by gel-filtration on Sephadex G-75 of nasal secretions from one patient from each group.

found in the fractions corresponding to SLPI-elastase complexes. In nasal secretions from the patient with allergic rhinitis, SLPI eluted in a single peak corresponding to the fractions where SLPI appears in a free form. Only a very small amount of granulocyte elastase was found in nasal secretions from the patient with allergic rhinitis (Figure 1). The amounts of α -1-PI were too low to be measurable in all three samples.

6. *Granulocyte elastase activity*

Granylocyte elastase activity was not found in any sample when tested with L-Pyroglutamyl-L-prolyl-L-valine-p-nitroanilide.

DISCUSSION

Virus rhinitis and allergic rhinitis are inflammatory conditions of the nasal mucous membrane. Previous studies have shown an increased content of proteins from granulocytes in nasal secretions from patients with allergic rhinitis i.e. eosinophilic cationic protein and myeloperoxidase (Linder et al., 1987). Like granulocyte elastase, myeloperoxidase is released from the primary granulae of granulocytes. In the present study we have examined granulocyte elastase and its inhibitors SLPI and α-1-PI and compared these proteins in nasal secretions from healthy persons and patients with various inflammatory conditions such as allergic rhinitis and common cold.

Due to different methods in collecting the nasal secretions, the contents of SLPI and granulocyte elastase were correlated to albumin. The ratio between SLPI and albumin in the different groups showed that there were relatively more SLPI in nasal secretions from healthy persons. Presumably the differences are due to an increased permeability of the mucosa vessels, causing leakage of albumin into nasal secretions in patients with allergic rhinitis and common cold. In several samples the amounts of α-1-PI were too low to be measurable. Granulocyte elastase was found in extremely low amounts in patients with allergic rhinitis, suggesting a minimal release of elastase from granulocytes in patients with this disease. This was further confirmed by the gel-filtration of nasal secretions. In all three groups SLPI was found in an active form and in excess of granulocyte elastase, which thus was completely inhibited. In contrast purulent maxillary secretions from patients with sinusitis have been shown to contain an excess of granulocyte elastase and free elastase activity (Fryksmark et al., 1985).

The differences in concentration of SLPI, α-1-PI and granulocyte elastase between the groups are not, even if significant, large enough to allow identification of the various conditions by these methods. However, the results indicate that SLPI is the main inhibitor in nasal secretions and that α-1-PI plays a minor role.

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