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Nasal neutrophil activity and mucinous secretory responsiveness in COPD

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Summary

Patients with chronic obstructive pulmonary disease (COPD) frequently report nasal symptoms. In the present study, we have examined whether or not COPD is associated with any nasal inflammation. Plasma exudation evoked by histamine challenges has been employed to improve the recovery of inflammatory indices in nasal lavage fluids. In 23 COPD-patients and 26 healthy subjects, all without history or signs of allergic rhinitis, nasal polyposis, or chronic rhinosinusitis, nasal saline-lavages were performed with and without histamine. α_2-Macroglobulin, fucose, eosinophil cationic protein (ECP) and myeloperoxidase (MPO) were determined as indices of plasma exudation, mucinous secretion, eosinophil activity and neutrophil activity, respectively. The difference in MPO-levels between the histamine and the saline lavage was greater in COPD patients compared with healthy subjects ($P<0{\cdot}05$). Also, COPD patients reporting nasal symptoms presented an increase in MPO at histamine challenge ($P<0{\cdot}05$, cf. saline) and greater differences in MPO and fucose, respectively, between the histamine and the saline lavage ($P<0{\cdot}05$, cf. patients without symptoms). We conclude that COPD is not associated with any marked nasal inflammation. However, our observation on increased MPO-levels at histamine challenge suggests some degree of increased neutrophil activity in this condition. Furthermore, when associated with nasal symptoms, COPD may be associated with an increased nasal secretory responsiveness.

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Introduction

A potential comorbidity of nasal and bronchial airways has been discussed throughout the medical history of respiratory diseases (Persson *et al.*, 1992). In modern times, the occurrence of nasal symptoms and inflammation in bronchial asthma has become widely recognized (Leynaert *et al.*, 2000; Palma-Carlos *et al.*, 2001), but only recently it has been suggested that also chronic obstructive pulmonary disease (COPD) may be associated with a significant nasal symptomatology (Montnemery *et al.*, 2001). In a questionnaire-based survey, nasal complaints were thus almost as common in self-reported COPD as in self-reported asthma (4). Interestingly, the COPD group was distinguished from those with asthma with regard to the nature as well as the triggering factors of nasal symptoms (Montnemery *et al.*, 2001). These observations suggested the possibility that the condition of the nose in COPD might differ from the eosinophilic inflammation that characterizes rhinitis in asthma and allergic airway diseases. However, almost nothing is known about nasal mucosal features in COPD.

The present study explores secretory, exudative and granulocyte activation indices of the nasal mucosa of patients with COPD (and of matched control subjects) with or without nasal symptoms. Our focus is on indices appearing in nasal lavage fluids at baseline and, particularly, after topical challenge with histamine. Histamine acutely causes plasma exudation responses in the airways, involving the egression of bulk plasma proteins from the microcirculation (Persson *et al.*, 1998; Greiff *et al.*, 2000a). After passing through the mucosal tissue, the proteinaceous plasma exudate moves between the epithelial lining cells into the airway lumen through a valve-like mechanism (Berg *et al.*, 2003), where it may be determined as increased levels of α_2-macroglobulin (Persson *et al.*, 1998). The induced exudation may also enrich the mucosal surface liquids with cell-derived mediators that would travel along with the plasma exudate from the mucosal tissue into the airway lumen (Persson *et al.*, 1998). In the present study, we have analysed nasal lavage fluid levels of eosinophil cationic protein (ECP) and myeloperoxidase (MPO) as indicators of eosinophil and neutrophil activity, respectively. We have also determined fucose in nasal lavage fluids to assess the occurrence of mucinous secretions (Greiff *et al.*, 2000b).

Material and methods

Study design

Patients with COPD were compared with healthy subjects matched for age and sex. The present study comprised two visits. At visit one, a physical examination was carried out. Also, the participants answered a questionnaire focusing on nasal and chest symptoms/diseases (Montnemery *et al.*, 2001). At visit two, nasal saline lavages with and without histamine were carried out. Levels of α_2-macroglobulin, fucose, ECP and MPO were measured as indices of plasma exudation, mucinous secretion, eosinophil activity and neutrophil activity respectively. The study was approved by the regional research ethics committee and informed consent was obtained.

Physical examination

Patients with COPD and healthy subjects had their medical history taken and received a physical examination that included anterior rhinoscopy and pulmonary auscultation. Skin prick tests were carried out including common air-borne regional allergens (ALK, Copenhagen, Denmark): Birch, grass, and mugworth pollens, house dust mites, moulds, as well as cat, dog and horse dander. Lung function tests were carried out using a spirometer (Vitalograph Alpha, Vitalogarph, Buckingham, UK). Particular care was taken to identify and exclude patients with allergic rhinitis, nasal polyposis and chronic rhinosinusitis. Also, subjects with a positive skin prick test were excluded. Furthermore, the participants had to be free of airway infections for a period of 4 weeks prior to the start of the study. Any medication with ipratropium bromide or β_2-agonists was discontinued 24 h prior to visit two, except short-acting β_2-agonists that were allowed up to 8 h prior to this visit. Medication with bronchial glucocorticoids was withdrawn 2 weeks prior to visit two, and patients on nasal or oral glucocorticoids were excluded.

Patients with COPD

Twenty-three patients with COPD (mean age 64 years, range 48–74 years, seven males) were recruited from and by a local general practitioner (Dr Nihlén, Västra Fäladen Primary Health Care Center, Landskrona). They were all current or ex-smokers with ten pack-years or more, and they had received a diagnosis of COPD at least 1 year prior to the study. None of the patients had experienced exacerbation of their airway condition for a period of 1 month prior to the study. The spirometry criteria for COPD was a ratio between forced respiratory volume in 1 s (FEV1) and vital capacity (VC) below 70% in combination with a FEV1 of <80% of the predicted normal value. In addition, it was required that the increase in FEV1 at 15 min after inhalation of 1·5 mg of terbutaline (Bricanyl Turbuhaler, AztraZeneca, London, UK) should be less than 15%, and less than 200 ml, compared with the initial value.

Healthy control subjects

Twenty-six airway/lung healthy subjects (mean age 58 years, range 45–65 years, nine males) were recruited from the same catchment area. These subjects all declared that they had not received a diagnosis of chronic bronchial/lung disease and that they did not experience chronic bronchial/lung symptoms. Their lung function was normal with an FEV1/VC ratio above 70% in combination with an FEV1 of >90% of the predicted normal value.

Nasal symptoms

Nasal symptoms were not specifically recorded prior to enrolment, and whether or not nasal symptoms were present at inclusion did not affect inclusion/exclusion unless signs at the anterior rhinoscopy and results of the skin prick test were suggestive of allergic rhinitis, upper respiratory tract infection, nasal polyposis, or chronic rhinosinusitis. Whether or not nasal symptoms were present in the study subjects was then derived from information obtained from a questionnaire focusing on nasal and bronchial/lung symptoms/diseases (Montnemery *et al.*, 2001). Accordingly, the subjects answered two questions: 1. 'Have you any nasal symptom?' and 2. 'What type of symptoms do you have?'. Alternatives given to the latter question included blockage, secretion, thick yellow secretion, sneezes and itching. The questionnaire was administered and answered after the subjects had been enrolled in the study.

Nasal lavage

A nasal pool device was used for saline lavage and for concomitant histamine and challenge and lavage of the nasal mucosa (Greiff *et al.*, 2001). The nasal pool device is a compressible plastic container equipped with a nasal adapter. The adapter is inserted into one of the nostrils and the device is compressed by the sitting subject leaning forward in a 60° flexed neck position. The pool fluid is thus instilled in one of the nasal cavities and maintained in contact with a large area of the mucosal surface for a determined period of time. When the pressure on the device is released, the fluid returns into the container. In the present study, the volume of the nasal pool fluid was 15 ml. Initially, two 30-s isotonic saline lavages were carried out in order to remove accumulated liquids surface and to create baseline conditions. A 10-min isotonic saline lavage was then carried out followed 10 min later by a 10-min histamine (400 $\mu g\ ml^{-1}$) challenge and lavage. The recovered lavage fluids were centrifuged (105 *g*, 10 min, 4°C) and aliquots were prepared from the supernatants and frozen (−20°C) for later analysis.

Analysis

α_2-Macroglobulin, ECP and MPO were analysed in supernatants as they were, whereas fucose was analysed in homogenized

aliquots of the supernatants. α_2-Macroglobulin was measured using a radioimmunoassay sensitive to 7·8 ng ml^{-1}. The intra- and inter-assay coefficients of variation are 3·8–6·0 and 3·1–7·2%, respectively. Fucose was measured using parallel ligand-exchange chromatography and fluorescence detection sensitive to 5·0 μM (Freney *et al.*, 2001). The intra- and inter-assay coefficients of variation are 15–25 and 20–35%, respectively. ECP was measured using a commercially available fluoroimmunoassay (Pharmacia Diagnostica, Uppsala, Sweden). MPO was measured using a commercially available enzyme-linkded immunosolvent assay (ELISA) kit (R & D Systems, Abingdon, UK).

Statistics

Differences in lavage fluid levels of α_2-macroglobulin, fucose, ECP, and MPO, respectively, between observations at baseline (saline) and at histamine challenge were examined using the Wilcoxon signed rank-test. Differences between patients with COPD and healthy subjects, as well as between subjects with and without nasal symptoms, were examined using the Mann–Whitney U-test. P-values <0·05 were considered statistically significant. Data are presented as mean ± SD.

Results

Patients with COPD presented an FEV1 (% of predicted) at 55·6 ± 16·1% and a FEV1/VC at 55·2 ± 13·2%. The corresponding figures for healthy subjects were 97·9 ± 13·2% and 77·4 ± 6·7%. Amongst the patients with COPD, 14 were smokers, 9 ex-smokers, and 0 never-smokers. The corresponding figurers for healthy subjects were 4, 9, and 13. Patients with COPD reported 28·8 ± 18·9 pack-years and healthy subjects 7·7 ± 12·1 pack-years.

Ten COPD patients and five healthy subjects reported recurrent or permanent nasal symptoms. Ten of these subjects were current smokers, four ex-smokers, and one a never-smoker. Nasal blockage was the most common symptom, being reported by seven patients with COPD and by three healthy subjects. Nasal secretion, thick yellow secretion, sneezes, and itching, respectively, was reported by seven, two, five, and zero of the patients with COPD and by one, zero, two, and zero of the healthy subjects.

Baseline levels of α_2-macroglobulin, fucose, ECP, and MPO did not differ between patients with COPD and healthy subjects (Table 1). The histamine-challenge increased the lavage fluid levels of α_2-macroglobulin (P<0·001, cf. saline), but the cellular indices were not significantly affected in these groups of subjects (Table 1). However, nasal lavage fluid levels of MPO exhibited a greater increase from baseline at histamine challenge in patients with COPD compared to healthy subjects (P<0·05) (Table 2). Histamine also produced an increase in lavage fluid levels of fucose, but this increase failed to reach statistical significance.

In the subgroup of COPD patients that exhibited nasal symptoms (n = 10) histamine increased nasal lavage fluid levels of MPO (P<0·05, cf. saline) (Table 3). Also, in these patients, nasal lavage fluid levels exhibited greater positive differences in both MPO and fucose levels between the histamine lavage and the saline lavage data than were detected in COPD patients without nasal symptoms (P-values<0·05) (Table 4). Subjects

Table 1 Levels of α_2-macroglobulin (α_2-M.), fucose, eosinophil cationic protein (ECP) and myeloperoxidase (MPO) in saline (baseline) and histamine lavages in patients with chronic obstructive pulmonary disease (COPD) and healthy subjects (mean ± SD).

	COPD patients		Healthy subjects	
	Saline	Histamine	Saline	Histamine
α_2-M. (μg ml^{-1})	0·1 ± 0·1	2·2 ± 3·5	0·2 ± 0·3	3·4 ± 4·5
Fucose (μM)	24·9 ± 24·7	50·3 ± 131·8	20·5 ± 17·0	43·8 ± 98·9
ECP (ng ml^{-1})	1·3 ± 1·0	1·4 ± 1·9	2·4 ± 2·8	2·1 ± 2·6
MPO (ng ml^{-1})	20·9 ± 39·3	46·8 ± 101·5	41·6 ± 60·9	36·7 ± 71·7

Table 2 Differences (between histamine and saline observations) in levels of α_2-macroglobulin (α_2-M.), fucose, eosinophil cationic protein (ECP) and myeloperoxidase (MPO), respectively, in patients with chronic obstructive pulmonary disease (COPD) and healthy subjects (mean ± SD). In patients with COPD, the difference in levels of MPO was significantly greater than in healthy subjects.

	COPD patients	Healthy subjects	*P*-value
α_2-M. (μg ml^{-1})	2·1 ± 3·4	3·2 ± 4·5	0·379
Fucose (μM)	25·4 ± 130·8	23·3 ± 98·2	0·270
ECP (ng ml^{-1})	0·1 ± 2·0	−0·2 ± 3·2	0·768
MPO (ng ml^{-1})	26·0 ± 87·6	−4·9 ± 74·3	0·048

Table 3 Levels of α_2-macroglobulin (α_2-M.) (μg ml^{-1}), fucose (μM), eosinophil cationic protein (ECP) (ng ml^{-1}), and myeloperoxidase (MPO) (ng/ml) in patients with chronic obstructive pulmonary disease (COPD) with nasal symptoms (n = 10) and in healthy subjects with nasal symptoms (n = 5) (mean ± SD). COPD-patients reporting nasal symptoms presented an increase in MPO at histamine challenge.

	COPD with nasal symptoms			Healthy with nasal symptoms		
	Saline	Histamine	*P*-value	Saline	Histamine	*P*-value
α_2-M.	0·1 ± 0·1	3·0 ± 3·4	0·003	0·4 ± 0·4	6·9 ± 8·0	0·068
Fucose	23·3 ± 26·6	89·4 ± 194·0	0·292	29·6 ± 18·4	39·8 ± 40·1	0·632
ECP	1·3 ± 0·7	1·9 ± 2·8	1·000	2·8 ± 3·0	2·0 ± 2·1	1·000
MPO	26·8 ± 48·6	67·0 ± 111·6	0·046	81·4 ± 99·3	46·4 ± 62·2	0·242

Table 4 Differences (between histamine and saline observations) in levels of α_2-macroglobulin (α_2-M.), fucose, eosinophil cationic protein (ECP), and myeloperoxidase (MPO), respectively, in patients with chronic obstructive pulmonary disease (COPD) with and without nasal symptoms (mean ± SD). In patients with nasal symptoms, the differences in levels of fucose and MPO, respectively, were significantly greater than in patients without nasal symptoms.

	With nasal symptoms	Without nasal symptoms	*P*-value
α_2-M. ($\mu g\ ml^{-1}$)	2·9 ± 3·6	1·5 ± 3·3	0·052
Fucose (μM)	66·1 ± 190·5	−5·9 ± 41·6	0·027
ECP ($ng\ ml^{-1}$)	0·6 ± 2·6	−0·3 ± 1·2	0·888
MPO ($ng\ ml^{-1}$)	40·1 ± 67·3	15·1 ± 101·8	0·031

without COPD did not exhibit the above differences between symptomatic and non-symptomatic nasal conditions.

Discussion

In agreement with previous survey findings (Montnemery *et al.*, 2001), a larger proportion of the present patients with COPD reported nasal symptoms compared with respiratory healthy subjects. As suggested by differences between histamine and saline challenge data on MPO and fucose levels in this study, individuals suffering from both COPD and nasal complaints exhibited greater nasal neutrophil activity and secretory responsiveness than COPD patients without nasal symptoms. Histamine and saline challenge data obtained from the entire group of patients with COPD similarly indicated greater increases in levels of MPO compared with healthy subjects. By contrast, nasal lavage fluid levels of ECP and α_2-macroglobulin did not differ between the subgroups. The present preliminary observations are of interest with regard to a potential nasal involvement in the diathesis of COPD as well as to the possibility that nasal mucosal processes, including neutrophil activity and mucinous secretory responses, in part may mimic bronchial processes in individuals with COPD.

Nasal symptoms were reported by 43% of the present COPD patients. The corresponding figure for healthy subjects was 19%. Relevant to patient inclusion, the nasal symptomatology in combination with signs at rhinoscopy and results of the skin prick test was neither distinctively suggestive of allergic rhinitis, upper respiratory tract infections, nasal polyposis, nor chronic rhinosinusitis. This likely reflects the particular care that was taken to identify and exclude such patients from the present study.

In the present study, there were no differences in saline lavage levels of either ECP or MPO between patients with COPD and healthy subjects. However, at histamine-challenges, a greater relative increase from baseline in nasal output of MPO was observed in patients with COPD than in healthy subjects. It is conceivable that the process of extravasation, lamina propria distribution, and luminal entry of bulk plasma, produced by the histamine challenge, successfully moved MPO from the tissue into the airway lumen, as previously has been demonstrated for other tissue solutes (Persson *et al.*, 1998). Inferentially, COPD may be characterized by some degree of on-going nasal neutrophil activity. Conversely, the lack of any nasal output of ECP at histamine-challenge suggests that COPD is not associated with increased nasal eosinophil activity.

Airway secretion is a defence mechanism in health and a pathological factor in disease (Rogers, 1994; Jeffery & Li, 1997; Jeffery, 1999; Rogers, 2000). For example, airway hypersecretion of mucus has been demonstrated as a major manifestation of COPD (Jeffery, 1999; Rogers, 2000). We have recently demonstrated that the nasal output of fucose, a common sugar moiety of the mucin molecule, is increased by common challenges such as methacholine, histamine, capsaicin and benzalkonium chloride as well as by allergen (allergic rhinitis) (Storaas *et al.*, 2000; Greiff *et al.*, 2000b). In the present subgroup of COPD patients that reported nasal symptoms, nasal lavage fluid levels exhibited greater differences in fucose levels between the histamine lavage and the saline lavage than detected in COPD patients without nasal symptoms. The observation suggests that a mucinous secretory hyperresponsiveness of the nasal mucosa characterizes COPD patients with nasal symptoms.

In the present study, there were no significant differences in nasal saline lavage levels of α_2-macroglobulin between patients with COPD and healthy subjects. α_2-Macroglobulin was employed as marker of plasma exudation, and we have previously demonstrated that this index represents bulk plasma (Persson *et al.*, 1998). Importantly, the luminal entry of plasma extends to threshold inflammatory responses (Persson *et al.*, 1998). Accordingly, it is unlikely that significant extravasation of plasma would have occurred in the present study without being reflected as increased levels of α_2-macroglobulin in the nasal lavage fluid samples. Hence, the present data suggest that COPD, and even nasal symptoms associated with COPD, may occur without exudative airway inflammation. By this feature, this condition would differ from inflammatory processes characterizing allergic rhinitis, common cold and nasal polyposis (Persson *et al.*, 1998).

COPD is usually associated with presence of CD8+ lymphocytes, increased neutrophil activity (Lacoste *et al.*, 1993; Keatings & Barnes, 1997; Pesci *et al.*, 1998; Balzano *et al.*, 1999; Jeffery, 1999; Rutgers *et al.*, 2000) and mucinous secretion (Jeffery, 1999; Rogers, 2000). The present observations suggest the possibility that COPD is also associated with a degree of nasal neutrophil activity and that nasal symptoms in COPD may be associated with neutrophil activity as well as an increased mucinous secretory responsiveness. These preliminary observations may, to some degree, suggest and define a 'pan-airway' condition in COPD. Further studies are warranted to address this possibility.

We conclude that COPD, and especially COPD with nasal symptoms, may be associated with some degree of increased neutrophil activity and a secretory (mucinous) hyperresponsiveness. However, this condition may not be associated with

any eosinophil activity or with any marked nasal neutrophilic or exudative inflammation.

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