Structure of central airways in current smokers and ex-smokers with and without mucus hypersecretion: relationship to lung function

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ABSTRACT Forty five patients who underwent thoracotomy and lung resection for tumour were studied to compare the structure of the central airways in current smokers and ex-smokers. The patients were divided into four groups: current smokers with mucus hypersecretion ($n = 15$), current smokers without mucus hypersecretion ($n = 14$), ex-smokers with mucus hypersecretion ($n = 5$), and ex-smokers without mucus hypersecretion ($n = 11$). Quantitative histological studies of the airway wall showed no difference in gland size, smooth muscle, connective tissue, or Reid index between the groups. The central airways of patients with mucus hypersecretion showed increased mucosal inflammation. The five ex-smokers in whom mucus hypersecretion persisted after they had stopped smoking had both central and peripheral airways affected by the inflammatory response, and these patients also had an abnormal result in the nitrogen washout test.

Introduction

Cigarette smoking has been identified as the major risk factor for development of chronic obstructive pulmonary disease. Changes in central airways associated with prolonged cigarette smoking include mucus hypersecretion and mucous gland enlargement.¹⁻⁴ The structural basis for mucus hypersecretion was thought to be enlargement of the bronchial glands, but a recent study has shown that it may be related to an inflammatory process in the central airways.⁵

The purpose of this study was to examine the effect of smoking habit on central airways structure and to determine whether the reduction in mucus hypersecretion associated with cessation of smoking was due to changes in severity of the inflammatory process in the airway wall or a reduction in size of the bronchial mucous glands. At the same time we attempted to determine whether persistence of mucus hypersecretion after cessation of smoking was associated with more severe airways disease. We used the standard clinical criteria for chronic bronchitis, con-

sisting of a history of a productive cough occurring on most days for at least three months in the year for at least two successive years. We have used the term mucus hypersecretion throughout to emphasise the clinical rather than pathological nature of the term.

Methods

PATIENTS

We studied 45 patients admitted to St Paul's Hospital, for resection of an upper lobe in 40 cases and a lung in five; in most this was for bronchogenic carcinoma. After the diagnosis had been made and surgery decided on, the study was explained to the patient and informed consent was obtained in all cases.

A modified British Medical Research Council questionnaire was used to assess cigarette smoking and cough and sputum history. An ex-smoker was defined as a subject who had ceased smoking at least four months before surgery. Mucus hypersecretion was defined as indicated in the introduction. There were 29 current smokers, 15 of whom had mucus hypersecretion; the ex-smoking group consisted of 16 patients, five of whom had mucus hypersecretion. In the group with mucus hypersecretion the patients had not been smoking for a mean of two (median 3.5) years. The mean interval since those in the group without mucus hypersecretion stopped smoking was

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Accepted 15 June 1987

10 (median 3) years (one patient was a long term ex-smoker of 45 years).

PULMONARY FUNCTION STUDIES

Pulmonary function tests were performed within the week before surgery with an air conditioned pressure compensated volume displacement body plethysmograph. Flow was measured with a Fleisch No 3 pneumotachometer coupled to a Sanborn 270 differential pressure transducer (Sanborn Co, Wallham, Montana) and volume was measured with a Krogh spirometer coupled to a linear displacement transducer (type 300 NR; Shaevitz Engineering, Pennsauken, New Jersey). Functional residual capacity (FRC) was determined by the Boyle's law technique,⁶ and residual volume (RV) and total lung capacity (TLC) were calculated. Forced expiratory volume in one second (FEV₁), forced vital capacity (FVC), and maximal flows at 50% (V̇max₅₀) and 25% (V̇max₂₅) of FVC were calculated from digitised flow and volume signals during forced expiratory manoeuvres. At least three expiratory efforts were produced and the forced expiratory manoeuvre with the largest sum of FEV₁ and FVC was selected. Single breath nitrogen washout was performed as described by Buist and Ross⁷ and the slope of phase 3 ($\Delta N_2/l$) was calculated. Diffusing capacity (transfer factor, TLCO) was measured by the steady state technique. Values were expressed as percentages of the predicted values according to the prediction formulae of Morris *et al*⁸ (FEV₁, FVC), Dosman *et al*⁹ (V̇max₅₀, V̇max₂₅), Buist and Ross⁷ ($\Delta N_2/l$), and Bates *et al*¹⁰ (TLCO).

MORPHOLOGICAL STUDIES

Surgically resected specimens were fixed in inflation with 10% formalin or 3% buffered glutaraldehyde by intrabronchial infusion at a constant pressure of 25 cm H₂O for 24 hours. They were then serially sliced in a sagittal plane.

Samples of five intrapulmonary cartilaginous airways from each specimen were selected and designated as "central" airways. Sections from areas showing bifurcation of airways, presence of tumour, or draining areas of obstructive pneumonitis were excluded. The sections were decalcified in 15% formic acid for 24 hours, processed for histological examination in the usual manner, and then cut at 5 µm thickness and stained with haematoxylin and eosin. All measurements in cartilaginous airways were performed with a camera lucida and an Apple II computer assisted digitising board. The luminal diameter of each cartilaginous airway was measured as the maximum distance perpendicular to the long axis of the airway lumen. The areas of the lumen and wall (gland, cartilage, smooth muscle, and connective tis-

sue) were measured by tracing each component and expressing it as a percentage of the total tissue area. Whenever possible, the Reid index was calculated for each airway by the method of Thurlbeck *et al*.³

The cartilaginous airways were graded by a modification of the pictorial grading method of Cosio *et al*.¹¹ Each airway was divided into quadrants and a separate grade was determined (from 0 to 3, normal to severe) for each quadrant according to the presence or absence and the severity of each of the following: mucosal (epithelial) inflammation, goblet cell metaplasia, squamous cell metaplasia, pigment deposition, and mural inflammation affecting each of glands, gland ducts, nerves, smooth muscle, and interstitium. The grading technique is subjective, and is based on a comparison of the amount and intensity of the feature being graded (for example, inflammation) on the slide with a standard set of photographs that illustrate each grade of each feature. A single score for each airway was then determined for each variable by summing the individual quadrant scores and expressing this total as a percentage of the maximum possible score. In addition, a score for inflammation of the cartilaginous airways was calculated as the sum of the six components, with the maximum possible score 600.

The pathology of the peripheral airways (membranous and respiratory bronchioles) was assessed by the method of Wright *et al*.¹² All airways had an internal diameter of under 2 mm. A membranous bronchiole was defined as an airway with a complete fibromuscular wall, while a respiratory bronchiole was defined as partially alveolated.

The midsagittal slice was used for estimating the degree of emphysema by a modification of the pictorial grading method of Thurlbeck *et al*.¹³

STATISTICAL ANALYSIS

The relationship of smoking habit to pulmonary function, airways disease, and emphysema was examined by dividing the patients into two groups: patients with current mucus hypersecretion who had had a productive cough occurring on most days for at least three months in the year for at least two successive preceding years and a control group who did not have these symptoms.¹⁴ These groups were then subdivided into current smokers and ex-smokers. A univariate one way analysis of variance was used to ascertain whether there were differences between groups.

Results

The effect of smoking habit on pulmonary function is shown in table 1. Flow rates and carbon monoxide diffusing capacity were similar in all groups. FRC was

Table 1 Pulmonary function data (mean (SD) percentages of predicted values)

	No mucus hypersecretion		Mucus hypersecretion	
	Current smoker	Ex-smoker	Current smoker	Ex-smoker
No of patients	14	11	15	5
Age (years)	59 (9)	61 (11)	59 (10)	63 (10)
Cigarette years*	837 (389)	1113 (937)	1061 (508)	1176 (1058)†
TLC	110 (18)	98 (13)	111 (13)	110 (7)
FRC	127 (22)‡	103 (20)	128 (22)‡	124 (18)
RV	147 (32)	117 (33)	141 (38)	137 (19)
FVC	95 (17)	93 (15)	99 (11)	98 (15)
FEV ₁	90 (20)	95 (16)	94 (19)	96 (20)
FEV ₁ /FVC	69 (9)	73 (6)	69 (10)	68 (9)
V _{max} ₅₀	48 (27)	62 (20)	60 (35)	62 (44)
V _{max} ₂₅	37 (24)	41 (20)	42 (28)	44 (30)
ΔN ₂ /l	232 (203)	198 (156)	157 (106)	303 (127)‡
TLCO	98 (39)	87 (30)	99 (46)	87 (18)

*Number of cigarettes per day multiplied by number of years smoked.

†p < 0.05 for difference from both groups of current smokers.

‡p < 0.01 for difference from ex-smokers without mucus hypersecretion.

TLC—total lung capacity; FRC—functional residual capacity; RV—residual volume; FVC—forced vital capacity; FEV₁—forced expiratory volume in one second; V_{max}₅₀, V_{max}₂₅—maximum flow at 50% and 25% of FVC; ΔN₂/l—slope of phase 3 of nitrogen washout curve; TLCO—transfer factor (diffusing capacity) for carbon monoxide.

increased in both groups of current smokers. Ex-smokers with mucus hypersecretion were slightly older than the other groups and had a greater lifetime cigarette consumption than current smokers.

There were no differences in Reid index or measurements of gland size, smooth muscle, cartilage, or connective tissue in the central airways between the groups (table 2). Patients with mucus hypersecretion showed greater mucosal inflammation than the ex-smokers without hypersecretion. Ex-smokers with mucus hypersecretion tended to have the most severe

mucosal inflammatory process with greater (p < 0.01) gland inflammation than the current smoking groups. The remaining inflammation parameters were similar in all groups. The current smokers had more goblet cell metaplasia than the ex-smokers. Ex-smokers with mucus hypersecretion had significantly greater squamous cell metaplasia than the other three groups.

The ex-smokers with mucus hypersecretion also had significantly greater membranous and respiratory bronchiole inflammation than the other groups (table

Table 2 Central airways data (means (SD))

	No mucus hypersecretion		Mucus hypersecretion	
	Current smoker	Ex-smoker	Current smoker	Ex-smoker
Structure*				
Gland %	4.0 (2.0)	3.5 (2.0)	4.0 (1.5)	5.7 (4.4)
Smooth muscle %	1.8 (0.6)	2.3 (1.3)	2.5 (1.1)	1.8 (0.3)
Cartilage %	18.2 (7.3)	17.9 (4.6)	20.1 (8.0)	20.0 (2.2)
Connective tissue %	76.0 (7.4)	76.2 (4.1)	73.4 (8.6)	72.6 (3.4)
Reid index	0.37 (0.09)	0.33 (0.11)	0.39 (0.08)	0.41 (0.09)
Wall				
Inflammation score				
Mucosa	44 (30)	34 (18)	59 (24)§	65 (14)§
Glands	27 (10)	31 (8)	28 (12)	39 (12)††
Gland ducts	18 (13)	23 (18)	24 (17)	17 (11)
Interstitium	14 (12)	21 (17)	21 (15)	26 (18)
Smooth muscle	2 (5)	1 (1)	3 (6)	4 (6)
Nerves	4 (3)	2 (4)	7 (8)	2 (2)
Total inflammation	110 (58)	112 (53)	141 (67)	152 (45)
Pigment	16 (6)	18 (10)	15 (10)	18 (8)
Epithelium				
Goblet cell metaplasia score	50 (27)§§	17 (17)	55 (25)§§	32 (11)‡
Squamous cell metaplasia score	5 (10)	0 (0)	1 (2)	13 (15)**

*% refers to percentage of total tissue area.

**p < 0.01 for difference from all three groups.

††p < 0.01 for difference from both current smoking groups.

‡p < 0.05 for difference from current smokers with mucus hypersecretion.

§p < 0.05 for difference from ex-smokers without mucus hypersecretion.

§§p < 0.01 for difference from ex-smokers without mucus hypersecretion.

3), as well as a more severe degree of emphysema and greater smooth muscle hypertrophy in the membranous bronchioles. Current smokers without mucus hypersecretion showed greater goblet cell metaplasia than ex-smokers without mucus hypersecretion and greater squamous cell metaplasia than current smokers with chronic bronchitis. Both groups of current smokers had more intraluminal macrophages than ex-smokers with chronic bronchitis.

Discussion

The data reported here (see table 2) show that central airways are composed of roughly 18–20% cartilage, 73–76% connective tissue, 4–5% glands, and 1–2% muscle, values that agree with those of previous reports.^{15–19} Although the relationship of smoking to mucous gland size is not well established, there is a trend for cigarette smokers to have larger glands.^{1–4} The inability to detect a decrease in either mucous gland size or the Reid index after cessation of cigarette smoking is consistent with the findings of Seltzer and colleagues.²⁰ They reported that in the recovery period after short term sulphur dioxide exposure gland acinar diameter, reflecting cellular activity, was partially reversible while gland acinar number, reflecting cellular proliferation, reverted slowly if at all. Mucous gland proportion may be a less sensitive measure of mucous gland size than either acinar diameter or acinar number and may therefore not reflect small but significant alterations in the mucous

secreting apparatus after smoking has stopped.

Goblet cell metaplasia is a non-specific response of the bronchial tree to irritants, and both animal and human studies show increases in number²¹ and degree of peripheral extension²² of goblet cells in response to cigarette smoke exposure. Seltzer and colleagues²⁰ described a mild to severe infiltrate of mononuclear cells around bronchi during chronic (6–18 months) exposure to sulphur dioxide, which was reduced in the recovery phase. These authors suggested that goblet cell metaplasia in the airway may be secondary to the inflammatory process, rather than a direct result of the inhaled insult. This concept is consistent with that put forward by Florey²³ concerning inflammatory processes of mucous membranes. In our study current smokers with mucus hypersecretion had the greatest values for central airway goblet cell metaplasia, and greater values for central airway mucosal inflammation than ex-smokers without hypersecretion. The central airway inflammatory process was also present in the ex-smokers who continued to have mucus hypersecretion and was accompanied by inflammation around the glands. In contrast, the ex-smokers who did not have mucus hypersecretion tended to have the least evidence of central airways inflammation.

The key feature of the peripheral airways data is that patients with mucus hypersecretion that persisted after they had stopped smoking showed evidence of a greater inflammatory response in both membranous and respiratory bronchioles. Although the number of

Table 3 Peripheral airways data (mean (SD) scores)

	No mucus hypersecretion		Mucus hypersecretion	
	Current smoker	Ex-smoker	Current smoker	Ex-smoker
MEMBRANOUS BRONCHIOLES				
<i>Wall</i>				
Inflammation	30(11)	26(13)	30(23)	48(8)*
Fibrosis	30(18)	29(22)	29(14)	37(10)
Smooth muscle	32(18)	22(11)	30(19)	43(23)§
Pigment	17(10)	18(10)	15(12)	14(11)
<i>Epithelium</i>				
Goblet cell metaplasia	24(18)	11(11)‡	15(13)	24(26)
Squamous cell metaplasia	17(12)	10(8)	9(9)‡	19(12)
RESPIRATORY BRONCHIOLES				
<i>Wall</i>				
Inflammation	21(10)	15(10)	20(16)	39(12)**
Fibrosis	23(14)	23(21)	24(15)	28(15)
Smooth muscle	12(10)	14(11)	17(16)	15(14)
Pigment	26(13)	26(15)	28(19)	29(18)
<i>Lumen</i>				
Macrophages	26(10)	22(13)	26(13)	12(5)†
Emphysema	13(12)	5(8)	14(13)	21(22)§

*p < 0.05 for difference from all three groups.

**p < 0.01 for difference from all three groups.

†p < 0.05 for difference from both current smoking groups.

‡p < 0.05 for difference from current smokers without mucus hypersecretion.

§p < 0.05 for difference from ex-smokers without mucus hypersecretion.

cases is small, these findings are consistent with the idea that mucus hypersecretion does not predict the presence of peripheral airways disease in current smokers, but they do suggest that patients whose mucus hypersecretion persists after they have stopped smoking may have an extensive inflammatory process affecting both the central and the peripheral airways.

The presence of increased central airway inflammation in mucus hypersecretion has not been well recognised. Previous studies²⁴⁻²⁷ have shown that airway inflammation is slight in mucus hypersecretion and does not distinguish those with hypersecretion from controls. Martin and coworkers,²⁸ using a grading scheme similar to ours, found a significant relationship between airway inflammation and the presence of cough but not sputum production. Central airway inflammation has also been associated with panlobular emphysema.²⁹ In this study, although the ex-smokers with mucus hypersecretion had significantly higher emphysema scores, it was mild and primarily centrilobular.

Peripheral airways inflammation has not generally been considered to be a component of mucus hypersecretion, although Matsuba and Thurlbeck³⁰ showed airway structural changes when lungs from patients with and without mucus hypersecretion were compared.

Previous studies have suggested that the rate of decline in pulmonary function decreases or that function may actually improve after cessation of smoking.³¹⁻³⁵ Although our study does not provide longitudinal data, it is of interest that those who had stopped smoking and continued to have mucus hypersecretion had a greater slope of phase 3 of the nitrogen washout curve ($\Delta N_2/l$) than any of the other groups. As the slope of phase 3 of the nitrogen washout response has recently been shown to predict the decline in FEV₁,³⁶ possibly patients with persistent mucus hypersecretion after cessation of smoking have a more severe inflammatory response in both central and peripheral airways that may ultimately lead to airways obstruction.

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