Respiratory epithelial permeability is unrelated to bronchial reactivity and small airway function in young smokers and nonsmokers

R.G. Taylor*, J.E. Agnew**, R.A. Francis***, D. Pavia*, S.W. Clarke*

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ABSTRACT: We studied eight young smokers and ten nonsmokers, to determine whether respiratory epithelial permeability to radiolabelled diethylenetriamine penta-acetate (99mTcDTPA) was related to small airway function or bronchial reactivity. Permeability was measured in inner (containing central airways) and outer lung zones by gamma camera. Lung-to-blood half-time (LB-T1) was corrected for blood background. Histamine was inhaled tidally (2 min inhalations) using doubling concentrations from 2 to 64 mg·ml⁻¹. Results of small airway function tests, and of bronchial reactivity (expressed as the threshold concentration (reducing forced expiratory volume in one second (FEV₁) by 2 SD), and as the percentage reduction in FEV₁ after histamine 16 mg·ml⁻¹) were similar in smokers and nonsmokers. LB-T1 was shorter in smokers than in nonsmokers in both inner (median (range) 21.5 (5.5-33) vs 63.5 (41-115) min; p<0.004) and outer (20.5 (5.5-30) vs 58.5 (39-105) min; p<0.004) zones. Neither inner nor outer zone LB-T1 was related to small airway function or bronchial reactivity. Bronchial reactivity and small airway tests may be abnormal in middle-aged smokers, but neither is related to the increased respiratory epithelial permeability of young smokers, in whom it appears too sensitive an index of airway integrity. Eur Respir J. 1988, 1, 319-323.

The reasons why chronic airflow obstruction develops in only a minority of smokers are largely unknown, and many studies have tried to identify those smokers who are particularly at risk. Sensitive tests of small airway function appear useful in this respect [31, 36]. More recently, other aspects of airway function have been examined in smokers. In particular, it has been shown that the permeability of the airways to the inhaled low molecular weight substance diethylenetriamine penta-acetate (DTPA) is increased in smokers [21], and that increased airway permeability caused by cigarette smoke is accompanied by increased bronchial reactivity in guinea pigs [20]. Furthermore, increased bronchial reactivity is present in almost a third of middle-aged smokers, in whom it is associated with a reduced forced expiratory volume in one second (FEV₁), and an accelerated rate of decline in FEV₁ [38]. These observations suggest that some relationship between different aspects of airway integrity may be relevant to the development of chronic airflow obstruction. We have looked for a relationship in smokers and nonsmokers between small airway function, bronchial reactivity, and respiratory epithelial permeability. We measured permeability selectively in inner and outer lung zones. The contribution to permeability of the conducting airways should be greater in the inner zone, so we looked here, particularly, for a relationship to bronchial reactivity. The combination of these three investigations of airway function has not been used in a single group of subjects before.

Subjects and methods

Subjects

Eighteen male hospital employees volunteered to be studied. They were of European extraction, aged 20-36 yr, and in good general health. Eight of them were regular smokers and ten were nonsmokers (had never smoked more than one cigarette a day for a year). None of the subjects took medication regularly or had had a respiratory infection within the previous eight weeks. Subjects were excluded if they had asthma. This was diagnosed by positive answers to enquiries about previous asthma or episodic wheeze, dyspnoea and tightness in the chest. All the subjects gave their written consent to be studied, and the study was approved by the hospital's ethical committee.

Lung function

The FEV₁ was measured with a Vitalograph spirometer. Maximal expiratory flow at 50% (Vmax50) and 25% (Vmax25) of vital capacity were
measured with an Ohio 840 spirometer and Bryans 60,000 X-Y recorder. An Ohio 700 nitrogen analyser was also used for the single-breath nitrogen test, to determine the slope of phase III (ΔN2/Δt) and closing volume as percentage of vital capacity, (CV/VC%). The largest of three values of FEV1, and the Vmax50, Vmax25, ΔN2/Δt and CV/VC% from the largest of three vital capacity tracings were each expressed as a percentage of the predicted value [5, 6, 25]. Results from the single-breath nitrogen test were not obtained from one smoker because of technical failure.

Bronchial reactivity

This was measured according to our modification [37] of an established protocol [7], using a Wright nebulizer which produced an aerosol mass median diameter (AMMD) of 3.45 (geometric sn 1.77) μm (Malvern 2600 Particle and Droplet Sizer, Malvern Instruments Ltd). Each subject inhaled nebulized solutions of histamine acid phosphate in doubling concentrations from 2–64 mg·ml⁻¹ through a short mouthpiece during 2 min of normal tidal breathing, until the strongest solution was inhaled, or the FEV₁ dropped by 20% (PC₂₀). Bronchial reactivity was assessed by determining the threshold concentration [14], the strength of histamine which reduced the initial FEV₁ by two standard deviations, derived from the three baseline plus the three post-saline values [37], and also as the percentage reduction in FEV₁ after inhaling nebulized histamine 16 mg·ml⁻¹ [37]. We used these indices rather than PC₂₀ to measure bronchial reactivity, because between them they give recordable values in all non-asthmatic subjects [8, 37], most of whom have a PC₂₀ greater than 16 mg·ml⁻¹ [15].

Respiratory epithelial permeability

This was measured according to a modification of the protocol of Jones et al. [21]. An aerosol of technetium-labelled diethylenetriamine penta-acectate (⁹⁹mTcDTPA) was generated from an Acorn nebulizer, shielded in a lead pot and driven at a compressed airflow of 10 l·min⁻¹. Nebulization continued until the aerosol generated filled a 25 litre reservoir bag, which was then left undisturbed for 5 min to allow large particles to settle out. The MMD of the aerosol subsequently inhaled was 0.6 (2.5) μm, with less than 5% of the particles >2 μm in diameter [12]. Each subject inhaled the aerosol with normal tidal breathing while seated in front of a gamma camera (International General Electric MaxiCamera). Inhalation was stopped when a predetermined lung count of 1,600 counts·sec⁻¹ had been reached. Sequential 1-min gamma camera images were then recorded, together with counts from a collimated scintillation counter positioned over the right thigh, pointing away from the bladder, to record the count as it built up in peripheral blood. After 30 min, a bolus of 8 MBq (approximately 220 μCi) of ⁹⁹mTcDTPA was injected intravenously to allow correction of the lung clearance curve for the contribution from vascular tissue in the lung detector.

Fig. 1. Inner and outer lung zones determined by gamma camera used for measuring respiratory epithelial permeability to ⁹⁹mTcDTPA. The outer margins of the lungs are defined by the 15% and 30% contours of a ventilation image obtained with ⁸¹Kr.
Results

There were no differences between the smokers and nonsmokers in age or baseline lung function (table 1). In the smokers, the median (range) duration of smoking was 9 (6–20) yr, and daily consumption 20 (10–30) cigarettes.

The corrected lung to blood half time (LB-T1/2) was significantly shorter in smokers than in nonsmokers in both inner and outer lung zones, but within each group of subjects, the LB-T1/2 was similar in the inner and outer zones (fig. 2).

In both smokers and nonsmokers, neither inner nor outer zone LB-T1/2 was significantly related to any index of small airway function ($V_{\text{max}50}$, $V_{\text{max}25}$, $\Delta N_2/\ell$, $CV/VC\%$) or bronchial reactivity (threshold concentration, percentage reduction in FEV1 with nebulised histamine 16 mg·ml$^{-1}$).

Representative illustrations of the results obtained from smokers are shown in figures 3–5.

Discussion

This study shows that, in young smokers with normal lung function, there is no relationship between respiratory epithelial permeability and either bronchial reactivity or the results of sensitive tests of small airway function. Previous studies have looked for such relationships, but have not compared permeability with both reactivity and small airway tests in the same subjects [10, 11, 24, 32, 33].

The lack of association between these three aspects of airway integrity is initially surprising, because smoking can certainly affect all three. Young smokers, whose spirometry is normal, may have small airway disease, the extent of which correlates with the functional abnormality expressed as the increase in $\Delta N_2/\ell$ and $CV/VC\%$ [9]. The primary lesion in such cases is a progressive inflammatory reaction in the

Table 1. - Age and results of baseline lung function tests in smokers and non-smokers. Values are median (range); lung function values are percent predicted.

<table>
<thead>
<tr>
<th></th>
<th>Age (yr)</th>
<th>FEV1 (l)</th>
<th>$V_{\text{max}50}$ (l/s)</th>
<th>$V_{\text{max}25}$ (l/s)</th>
<th>$\Delta N_2/\ell$ (l)</th>
<th>CV/VC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td>27 (20-36)</td>
<td>99 (83-121)</td>
<td>99 (49-114)</td>
<td>75 (36-87)</td>
<td>143 (106-187)</td>
<td>104 (66-159)</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>23 (20-29)</td>
<td>102 (77-114)</td>
<td>87 (61-112)</td>
<td>85 (53-107)</td>
<td>144 (84-198)</td>
<td>86 (38-149)</td>
</tr>
</tbody>
</table>

Smokers v. non-smokers: all p values >0.05.
Radioabnormal values of middle-aged smokers [13]. CV / VC% of functionality measured using volume curves, even when the smokers are similar to related to either the normal values of reactivity (as assessed by partial expiratory bronchial reactivity measured using volume curves). Even though permeability may reflect events taking place predominantly in the alveoli, whereas that of bronchial reactivity reflects changes in the conducting airways. Current methods of imaging cannot distinguish precisely where aerosol is deposited in the respiratory tract, the planar image being only two-dimensional [1] and acquisition time for tomographic images long, compared with the expected LB-T1. Commonly used techniques employ particle sizes and modes of inhalation which cause the DTPA aerosol to be deposited in the alveoli and small conducting airways, and large airway labelling is not seen [21, 22, 28]. In addition, the surface area of the respiratory tract increases enormously distal to the terminal bronchioles, so the alveolar influence on permeability predominates.

It is not certain if the permeability of the conducting airways is the same as that of the alveoli. In one study [11], subjects inhaled labelled DTPA aerosol, of aerodynamic mass median diameter 6.3 μm, rapidly to accentuate deposition on the central airways, and its subsequent rate of disappearance was similar to that reported by others who used 2 μm particles [21]. However, recent work suggests that mucociliary clearance, rather than epithelial permeability, may account for removal of much of the aerosol from the central airways [3]. We tried to allow for any regional difference in permeability and the fact that the particle size of the DTPA aerosol was smaller than that of the histamine aerosol by measuring permeability in an inner lung zone, which contained the central airways. Despite this, no relationship to reactivity emerged, and others have found similar results [32]. However, although the inner zone provided counts from the central airways, it also included alveoli lying in front of and behind them, because the image was only two-dimensional. So even though permeability appeared to be similar in the inner and outer zones within each of our two groups of subjects, this may not actually be the case.

It is unlikely that the larger particle size of the aerosol influenced the bronchial reactivity results, because reactivity is similar when smaller particles are used [34].

Even though smoking causes abnormalities of small airway tests, respiratory epithelial permeability and bronchial reactivity, there are several theoretical explanations for our observation that these indices of airway function are not related to one another in individual subjects. Firstly, the measurement of permeability may reflect events taking place predominantly in the alveoli, whereas that of bronchial reactivity reflects changes in the conducting airways. Current methods of imaging cannot distinguish precisely where aerosol is deposited in the respiratory tract, the planar image being only two-dimensional [1] and acquisition time for tomographic images long, compared with the expected LB-T1. Commonly used techniques employ particle sizes and modes of inhalation which cause the DTPA aerosol to be deposited in the alveoli and small conducting airways, and large airway labelling is not seen [21, 22, 28]. In addition, the surface area of the respiratory tract increases enormously distal to the terminal bronchioles, so the alveolar influence on permeability predominates.

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Several studies which used the inhaled 99mTe-DTPA method have confirmed the original observations of Jones et al. [21] that respiratory epithelial permeability is greater in symptomless smokers than in nonsmokers [18, 24, 28, 32]. There is good evidence that the increase in permeability is closely related to smoking [17, 23, 28-30].

Although bronchial reactivity measured using FEV1 is not increased in symptomless young smokers [4, 16, 27, 37], their small airways do show abnormal reactivity (as assessed by partial expiratory flow-volume curves), even when the smokers are similar to nonsmokers in pre-challenge function and in reactivity measured using FEV1 [27]. However, the degree of bronchial reactivity measured using FEV1 was not related to either the normal values of ΔN3/II or CV/VC% in young smokers [24, 37], or to the abnormal values of middle-aged smokers [13].

**Fig. 4.** Relation in smokers between outer zone corrected LB-T1 and Vmax50 (as percent predicted).

**Fig. 5.** Relation in smokers between outer zone corrected LB-T1 and ΔN3/II (as percent predicted).

**References**


38. Taylor RG, Joyce H, Cross E, Holland F, Pride NB. - Bronchial reactivity to inhaled histamine and annual rate of decline in FEV1 in male smokers and ex-smokers. Thorax, 1985, 40, 9–16.

RÉSUMÉ: Huit jeunes fumeurs et dix non fumeurs ont été étudiés pour déterminer la relation éventuelle entre d’une part la perméabilité épithéliale et d’autre part la fonction des petites voies aériennes ou la réactivité bronchique. La perméabilité était mesurée par une caméra dans les zones centrales (incluant les voies aériennes centrales) et périphériques. La demi-vie poumon-sang (LB-T1) était corrigée pour le bruit de fond d’origine sanguine. L’histamine était inhalée pendant 2 minutes de respiration normale, avec un doublement des concentrations de 2 à 64 mg/ml. Les tests explorant les petites voies aériennes et la réactivité bronchique (réduction de FEV1, pour la dose d’histamine de 16 mg/ml, exprimée en % de la valeur initiale) étaient semblables chez les fumeurs et les non fumeurs. Par contre LB-T1 était plus court chez les fumeurs que chez les non fumeurs tant dans les zones centrales (médiane étendue des variations) 21 (5-53) versus 63.5 (4-113) min, p < 0.004) que périphérique (20.5 (5.5-30) versus 58.5 (39-105) min, p < 0.004). LB1, qui concerne les zones centrales ou périphériques, n’est pas lié aux petites voies aériennes ni à la réactivité bronchique. La réactivité bronchique et les tests explorant les petites voies aériennes peuvent être anormaux chez les fumeurs d’âge moyen, mais ni l’une ni les autres ne sont liés à l’augmentation de la perméabilité épithéliale observée chez les jeunes fumeurs qui apparaissait lors être un index trop sensible d’intégrité des voies aériennes.