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*

ABSTRACT: We studied eight young smokers and ten nonsmokers, to determine whether respiratory epithelial permeability to radiolabelled diethylenetriamine penta-acetate (DTPA) 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-Tt) 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-Tt was shorter in smokers than in nonsmokers in both inner (median (range) 21 (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-Tt 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.

Received: March 12, 1987; accepted after revision August 17, 1987.

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 60000 X-Y recorder. An Ohio 700 nitrogen analyser was also used for the single-breath nitrogen test, to determine the slope of phase III ($\Delta N_2/\Delta t$) and closing volume as percentage of vital capacity, (CV/VC%). The largest of three values of FEV$_1$, and the $V_{\text{max}25}$, $V_{\text{max}50}$, $\Delta N_2/\Delta t$ 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.

**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-acetate ($^{99m}$TcDTPA) 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 $^{99m}$TcDTPA was injected intravenously to allow correction of the lung clearance curve for the contribution from vascular tissue in the lung detector.
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-Tt) was significantly shorter in smokers than in nonsmokers in both inner and outer lung zones, but within each group of subjects, the LB-Tt was similar in the inner and outer zones (fig. 2).

In both smokers and nonsmokers, neither inner nor outer zone LB-Tt was significantly related to any index of small airway function (Vmax 50, Vmax 25, ΔN2/L, 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 ΔN2/L 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</th>
<th>FEV1</th>
<th>Vmax 50</th>
<th>Vmax 25</th>
<th>ΔN2/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.
small airways [9]. Cigarette smoke causes a dose-
dependent inflammatory reaction in the airways of 
guinea pigs. This is matched in time and extent by an 
increase in respiratory epithelial permeability, and 
also in bronchial reactivity, perhaps caused by the 
release of nerve endings lying within the epithelium 
[19, 20, 35]. Intraluminal nerves may be similarly 
affected in man [26].

Several studies which used the inhaled \(^{99m}\)TcDTPA 
method have confirmed the original observations of 
Jonas 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 
FEV\(_1\) 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 FEV\(_1\) [27]. However, the degree of 
bronchial reactivity measured using FEV\(_1\) was not 
related to either the normal values of \(\Delta N_{31}/l\) or 
CV/VC% in young smokers [24, 37], or to the 
abnormal values of middle-aged smokers [13].

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 
independent individuals. Firstly, the measurement of 
permeability may reflect events taking place predomi-
nantly 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-T\(_2\). 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 bronchi-
oles, 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 \(\mu\)m, rapidly to accentuate deposition on the 
central airways, and its subsequent rate of disappear-
ance was similar to that reported by others who used 
2 \(\mu\)m 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 
histamine aerosol influenced the bronchial reactivity 
results, because reactivity is similar when smaller 
particles are used [34].

References
1. Agnew JE, Pavia D, Clarke SW. – Airways penetration of 
inhaled radioaerosol: an index to small airways function? Eur J 
2. Agnew JE, Bateman JRM, Pavia D, Clarke SW. – 
Radioactive demonstration of ventilatory abnormalities in mild asthma. 
– The relative permeabilities of human conducting and terminal 
airways to \(^{99m}\)Tc-DTPA. Eur J Respir Dis, 1987, 71, (suppl 153), 
68–77.
Predicted values for closing volumes
Quantitative analysis of the alveolar Bronchi al
The use of tests
Increased alveolar epithelial permeability in cigarette
Rapidly
Changes in the normal maximal expiratory flow-volume curve