Effects of Smoking on Bronchial Clearance of Technetium-99m-DTPA and Indium-113m-DTPA

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In asymptomatic smokers, epithelial permeability in the distal lung regions is increased. To date, the effect of smoking on the epithelial permeability in proximal lung regions is still debated. The measurement of bronchial clearance of inhaled radiolabeled diethylene-triaminepentaacetic acid (BC-DTPA) can be used to assess epithelial permeability of proximal bronchi, but there are two potential limitations to this method: in vivo breakdown of ^{99m}Tc-DTPA in smokers and mucociliary transport of DTPA. **Methods:** Eight nonsmokers and eight asymptomatic smokers were studied. We used a spinning disk system to generate an aerosol of large particles of ^{99m}Tc-DTPA or ^{113m}In-DTPA (MMAD 6.3 μm). To measure the bronchial clearance of ^{99m}Tc-DTPA and ^{113m}In-DTPA, we analyzed the perihilar regions of the lung. To determine the contribution of mucociliary transport, we measured the activity over a tracheal region of interest (ROI) in eight nonsmokers. **Results:** Technetium-99m-DTPA bronchial clearance did not differ in smokers (1.16 ± 0.54 %/min; mean ± s.d.) or nonsmokers (1.29 ± 0.51 %/min; ns). The ^{113m}In-DTPA bronchial clearances in nonsmokers (1.24 ± 0.51 %/min) and in smokers (1.01 ± 0.66 %/min) were similar to the ^{99m}Tc-DTPA bronchial clearances (ns). In the tracheal ROI, we found no increase in activity. **Conclusion:** In smokers, BC-DTPA was not increased compared to nonsmokers. In contrast to distal lung regions, there was no evidence of breakdown of the ^{99m}Tc-DTPA complex in the proximal regions of smokers' lungs. Mucociliary clearance does not significantly contribute to BC-DTPA.

Key Words: cigarette smoke; airway epithelial permeability; DTPA aerosol scintigraphy

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The respiratory epithelial clearance of ^{99m}Tc-diethylene-triamine pentaacetate (^{99m}Tc-DTPA) is commonly used to assess the permeability to solutes of the epithelium of terminal respiratory units (*1*). In asymptomatic smokers, several studies have shown that the respiratory clearance of ^{99m}Tc-DTPA is higher than in controls (*2,3*). Part of this increase can be attributed to chemical dissociation of the ^{99m}Tc-DTPA complex (*4*). The respiratory clearance of a more stable complex ^{113m}In-DTPA is still increased in smokers compared to nonsmokers (*4*).

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Animal studies suggest that smoking induces an increase in the permeability of the bronchial epithelium (*5–7*). Several attempts have been made to measure the bronchial epithelial clearance of aerosolized ^{99m}Tc-DTPA (BC-DTPA) in humans and in animals using particles of various sizes, ranging from 0.5 to 6 μm (*8–11*). These studies, however, include a significant portion of distal sites of clearance since there is peripheral deposition (especially in those studies that use particles <2 μm) and they all select areas of interest that cover the whole lung. We have developed a technique of measuring BC-DTPA that consists primarily of using a spinning disk system to generate large particles to ensure a predominantly proximal deposition and in selecting proximal regions of interest (ROIs) for analysis. With this technique, we found an increase in BC-DTPA in acute, but not chronic, asthma and in chronic obstructive lung disease (*12*).

The aim of this study was to compare the in vivo permeabilities of proximal bronchi to solutes in smokers and nonsmokers. The measurement of BC-DTPA, however, has two potential limitations. First, the increase in respiratory clearance of ^{99m}Tc-DTPA in smokers is partly related to oxidative breakdown of the ^{99m}Tc-DTPA complex (*4*) and second, the mucociliary transport may play a significant role in BC-DTPA (*8*). To determine whether breakdown of ^{99m}Tc-DTPA occurred in the proximal lung regions of smokers, we also measured the bronchial clearance of the more stable complex ^{113m}In-DTPA. To assess the contribution of mucociliary clearance, we measured the course of activity over a tracheal ROI.

MATERIALS AND METHODS

Subjects

Two groups of eight subjects were studied: one group of asymptomatic nonsmokers and one group of asymptomatic smokers. To be considered a smoker, a subject had to smoke more than 10 cigarettes a day. All subjects were free of respiratory symptoms and had no recent history of respiratory disease. Each subject had two measurements of BC-DTPA using ^{99m}Tc-DTPA or ^{113m}In-DTPA. Anthropometric data and pulmonary function tests of all subjects are shown in Table 1. All subjects provided written informed consent.

Lung Volume Measurement

Forced expiratory volume in 1 sec (FEV_1) and vital capacity (VC) were measured using a spirometer in the sitting position.

TABLE 1
Means and Standard Deviations of Age and Pulmonary Function Data in Smokers and Nonsmokers

	Nonsmokers (n = 8)	Smokers (n = 8)
Age (yr)	28.0 ± 7.8	27.0 ± 4.4
TLC (% pred)	100.0 ± 13.0	98.5 ± 12.6
VC (% pred)	100.5 ± 8.3	103.1 ± 9.1
FEV_1 (% pred)	108.0 ± 12.3	104.1 ± 12.6
FEF_{25-75} (% pred)	144.4 ± 34.8	98.5 ± 35.6

Total lung capacity (TLC) was derived from measured functional residual capacity performed by multiple-breath helium dilution. Results were expressed as a percentage of predicted values from the European Coal and Steel Community Survey (*13*).

Radioactive Aerosols

To generate the ^{99m}Tc-DTPA aerosol, 1.85 GBq (50 mCi) [^{99m}Tc]pertechnetate were eluted from a ^{99}Mo generator and introduced into a vial containing 9.1 mg DTPA-$CaNa_3$. Saline was added to the ^{99m}Tc-DTPA solution (MW 492 daltons) to a total volume of 10 ml. The binding of ^{99m}Tc to DTPA was analyzed using paper chromatography within 1 hr after preparation and found to be greater than 95% bound (n = 3). Indium-113m-DTPA (MW 504 daltons) was obtained from the labeling of 13 mg DTPA with 0.74 GBq (20 mCi) ^{113m}In. Indium-113m was eluted from a ^{113}Sn generator and was added to 10 ml saline. The radiopharmaceutical preparations were aerosolized immediately.

Measurements of Bronchial Clearance of DTPA

Our technique has been previously described (*12*). To obtain a deposition of particles mostly in the proximal bronchi, we used a spinning disk system (*14*) to produce an aerosol of large particles (MMAD, 6.3 μm; GSD, 2.1). The generator included a spinning disk of 2.5 cm in diameter, driven by a high-pressure air supply. The radioactive aerosol preparation was supplied from a nearby reservoir via a fine needle to the disk center at a rate of one drop per 4 sec. The spinning disk was enclosed in a glass bell jar 40 cm in height and 20 cm in diameter surrounded by removable 2-mm lead plates acting as radiation shielding. The bell jar was connected to a plastic tubing with a mouthpiece through which the patient inhaled while air entered through a second opening at the top of the bell jar. Air was exhaled through another plastic tubing with a one-way valve and a filter trap.

Subjects were seated facing the camera and breathed quietly at their resting tidal volume and frequency. They inhaled the aerosol until depletion of the radiolabeled solution, which lasted about 10 min. Care was taken to ensure that none of the subjects coughed during the 30-min observation. Radioactivity counts were acquired in 30-sec frames over the entire thorax in the posterior projection using a gamma scintillation camera and a high-energy collimator. When the subjects inhaled the ^{99m}Tc-DTPA aerosol, the thyroid area was monitored at the end of acquisition to check for activity which would be caused by fixation of [^{99m}Tc]pertechnetate. No significant increase in activity was found arguing against formation of [^{99m}Tc]pertechnetate.

Perihilar regions of the lungs were selected for analysis as previously described, focusing on the areas where deposition is predominant (*12*). Counts were corrected for radionuclide decay and plotted on a semi-logarithmic scale against time from peak radioactivity until the end of the experiment. The regression line of the counts was determined by the least square method during the 10-min period following peak activity in the ROI. The clearance of labeled DTPA was the negative slope of this line, expressed as the percent decline in activity per minute.

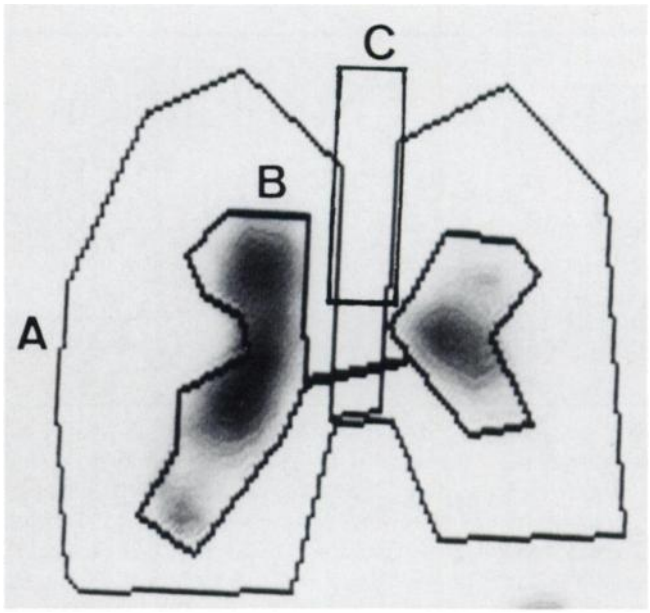

FIGURE 1. Deposition pattern of ^{113m}In-DTPA aerosol in both lungs (A), central airway (B) and tracheal (C) ROIs.

To assess the contribution of mucociliary transport to the measurement of BC-DTPA, we selected a ROI covering the trachea from the carina to 2–5 cm below the larynx (Fig. 1). This ROI included most of the trachea and the first centimeters of the main bronchi. We measured the change in activity of labeled DTPA over this ROI during the 10-min of BC-DTPA measurement; this measurement was made only in nonsmokers because of the potentially depressing effect of tobacco smoke on mucociliary clearance (*15*). The change in tracheal activity is expressed as %/min.

Statistical Analysis

Results are expressed as mean ± s.d. Functional data and clearance values were compared using analysis of variance or paired Student's t-test. A p value < 0.05 was considered significant.

RESULTS

Pulmonary function test results for the two groups of subjects are shown in Table 1. There was no difference in TLC, VC and FEV_1, but FEF_{25-75} was lower in smokers than in nonsmokers ($p < 0.05$).

The means and s.d. of the bronchial clearance of ^{99m}Tc-DTPA and ^{113m}In-DTPA in smokers and nonsmokers are listed in Table 2. Individual values are shown in Figure 2. There was no significant difference in the bronchial clearances of ^{99m}Tc-DTPA and ^{113m}In-DTPA in smokers compared to nonsmokers. In addition, in both smokers and nonsmokers, there was no significant difference between the bronchial clearance of ^{99m}Tc-DTPA or ^{113m}In-DTPA.

To assess the contribution of mucociliary transport to DTPA clearance, we measured the tracheal activity of DTPA in nonsmokers. In one subject, swallowing of radioactive saliva during activity recording made it impossible to reliably measure tracheal clearance. As shown in Table 3, activity over the tracheal region just after inhalation was low. For ^{99m}Tc-DTPA, background activity was 20.0 ± 17.6 cts/30 sec and peak activity was 135.1 ± 73.9 cts/30 sec (n = 7); for ^{113m}In-DTPA, background activity was 16.8 ± 29.4 cts/30 sec

TABLE 2
Means (%/min) and Standard Deviations of the Bronchial Clearances of Technetium-99m-DTPA and Indium-113m-DTPA in Nonsmokers and Smokers

	Nonsmokers (n = 8)	Smokers (n = 8)
^{99m}Tc-DTPA	1.29 ± 0.51	1.16 ± 0.54
^{113m}In-DTPA	1.24 ± 0.51	1.01 ± 0.66

and peak activity was 77.8 ± 72.0 cts/30 sec (n = 8). The changes in activity of ^{99m}Tc-DTPA and ^{113m}In-DTPA in nonsmokers in the tracheal region are listed in Table 3. The values are not significantly different from zero.

TABLE 3
Ratio of Activity over Tracheal Region-to-Background Activity after Inhalation of Radiolabeled DTPA and Change in Tracheal Activity after Inhalation of Technetium-99m-DTPA and Indium-113m-DTPA in Nonsmokers

	^{99m}Tc-DTPA (n = 7)	^{113m}In-DTPA (n = 8)
Ratio of activity to background activity	20.35 ± 23.80 (1.94–58.13)	10.32 ± 11.04 (2.76–35.91)
Change in tracheal activity (%/min)	0.56 ± 3.11 (−4.01 ± 6.13)	−0.50 ± 3.62 (−6.42 ± 5.39)

*Values in parentheses are mean ± s.d. and range.

DISCUSSION

In contrast to the respiratory clearance, smoking does not increase bronchial clearance of DTPA nor does it induce chemical breakdown of ^{99m}Tc-DTPA in the proximal airways. In addition, we found no increase in tracheal activity during BC-DTPA measurement, indicating that the mucociliary transport does not significantly affect the measurement of BC-DTPA.

To ensure that we measured in vivo bronchial clearance rather than respiratory clearance, we used a spinning disk system to generate an aerosol of large particles. Visual assessment of the aerosol deposition clearly demonstrates that predominantly central deposition was achieved (*12*). Peripheral deposition of the radiolabel, however, cannot be totally avoided, and some absorption from the peripheral regions of the lung inevitably occurred. In their evaluation of the bronchial clearance of DTPA in stable asthmatics, Elwood et al. (*9*) estimated that the peripheral deposition represented almost half of the central deposition. In the study by Kennedy et al. (*10*), visual representation of the aerosol distribution shows that deposition of particles did not predominate in central regions of the lung. Yet, in these studies, data were analyzed from the whole lung fields and reflect a combination of both the bronchial and the respiratory clearance of DTPA. By contrast, to minimize the contribution of peripheral absorption, we selected as areas of interest the perihilar regions of the lungs only, which contain relatively more conducting bronchi and is where most particles are actually deposited.

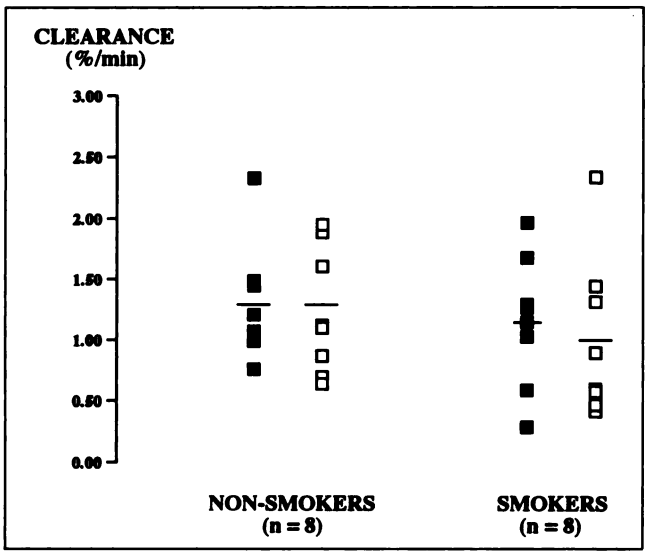

FIGURE 2. Individual values of bronchial clearances of ^{99m}Tc-DTPA (filled squares) and ^{113m}In-DTPA (open squares) in nonsmokers and smokers. Means are indicated by a bar.

Studies in asymptomatic smokers have shown that respiratory clearance of DTPA is increased (*2,3*), but part of this increase may be explained by dissociation of the ^{99m}Tc-DTPA complex with formation of [^{99m}Tc]pertechnetate which has a much more rapid clearance. This hypothesis was based on the fact that respiratory clearance of ^{113m}In-DTPA in smokers is significantly lower than that of ^{99m}Tc-DTPA (*4*). Based on the difference between the respiratory clearances of ^{99m}Tc-DTPA and ^{113m}In-DTPA, it has been estimated that dissociation of ^{99m}Tc-DTPA may account for up to 25% of the increase observed in smokers (*4*). A significant contribution of dissociation of the ^{99m}Tc-DTPA complex, however, was not supported by other studies, in particular urinalyses for [^{99m}Tc]pertechnetate (*16*). Nevertheless, even though dissociation of ^{99m}Tc-DTPA is at most small, the respiratory clearance of ^{113m}In-DTPA probably more acurately reflects actual respiratory clearance of DTPA in smokers. Our study did not find evidence that smoking does increase the bronchial clearance of ^{99m}Tc-DTPA, nor did we find a significant difference between the bronchial clearances of ^{99m}Tc-DTPA and ^{113m}In-DTPA.

DTPA can be cleared by transepithelial transfer or mucociliary transport. To date, the contribution of mucociliary transport to this process has not been conclusively determined. Bennett and Ilowite (*8*) calculated the rate of ^{99m}Tc-DTPA transfer across the bronchial epithelium by correcting ^{99m}Tc-DTPA bronchial clearance by mucociliary clearance, measured by the rate of mucociliary transport of ^{99m}Tc-albumin. This correction relies on the unproved assumption that ^{99m}Tc-DTPA, a small compound with a molecular weight of 492 daltons, has the same mucociliary clearance as human serum albumin, which has a much larger molecular weight of 66,000 daltons and different physico-chemical properties. In particular, there is no evidence that albumin and DTPA bind similarly to mucus. Furthermore, these authors used a polydisperse aerosol with a MMAD of 2.0 μm, which is likely to deposit in the peripheral regions of the lungs. Bronchial dep-

osition was enhanced by breathing maneuvers and delivery of the aerosol during the last half of inspiration, but peripheral deposition occurred and the investigators included the whole right lung field in their analysis. Finally, Bennett and Howite claimed that they observed labeled DTPA clearing from the lung into and up the trachea. We did not observe such a phenomenon in our subjects. If mucociliary clearance played a significant role in the bronchial clearance of DTPA, we should observe an increase in activity over the tracheal region in nonsmokers since:

1. In normal subjects, the rate of mucus transport in the trachea is about 5 mm/min.
2. In the study by Bennett and Ilowite (*8*), radioactivity appeared in the trachea within 15 min.
3. In our study, just after inhalation of radiolabeled DTPA, the activity over the trachea was low (which should improve the detection of an increase in activity).

We measured the activity over the tracheal region and found no increase during the 10 min after inhalation. This observation confirms that of Greiff et al. (*17*) who directly deposited ^{99m}Tc-DTPA in the trachea of anesthetized guinea pigs and observed no transport of the radiolabel above the initial level of deposition. Furthermore, smokers usually have depressed mucociliary clearance (*15,18*), but we failed to find decreased BC-DTPA clearance in smokers. We also used the same technique and found a dramatic increase in the bronchial clearance of DTPA during acute attacks of asthma (*12*), although mucociliary clearance in asthma is not increased (*19,20*). Although we cannot rule out the contribution of mucociliary transport to the clearance of DTPA, we found no indication of its significance, at least during the timeframe of our measurement.

In our sample of young asymptomatic smokers, DTPA clearance of the proximal portion of the bronchial tree is likely to be less affected by cigarette smoke than the distal portion for two reasons. First, the component of tobacco smoke responsible for the increase in the respiratory clearance of DTPA is contained in the particulate phase rather than the gas phase (*21,22*). The particulate phase comprises particles from 0.1 to 1 μm MMAD; the less hygroscopic of these particles are likely to deposit in peripheral bronchi and terminal respiratory units rather than in central bronchi (*23,24*) and therefore should not affect BC-DTPA in smokers. Second, pathological and lung function studies have shown that smoking-induced bronchial changes first appear in the distal bronchi (*25,26*). The pathological changes include inflammatory infiltration and epithelial damage, which in turn may mediate the effects of cigarette smoke on the respiratory clearance of DTPA.

CONCLUSION

This study shows that in vivo bronchial clearance of DTPA, in contrast to the respiratory clearance of DTPA, is similar in nonsmokers and asymptomatic smokers. This difference illustrates that the site of aerosol deposition may be an important determinant in the measurement of the clearance of aerosolized DTPA in lung diseases.

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