Clearance of ^{99m}Tc DTPA from guinea pig nasal, tracheobronchial, and bronchoalveolar airways

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Abstract

Technetium-99m labelled diethylenetriamine penta-acetate (^{99m}Tc-DTPA) was used to compare small solute absorption (clearance) from nasal, tracheobronchial, and bronchoalveolar airways in anaesthetised guinea pigs. ^{99m}Tc DTPA dissolved in saline was superfused through nasal and orolaryngeal catheters on to nasal and tracheobronchial airways; a small particle aerosol of nebulised ^{99m}Tc DTPA was delivered to the bronchoalveolar airways through a tracheostomy. Radioactivity over the appropriate region was then determined with a gamma camera. Mucociliary transport of ^{99m}Tc DTPA appeared not to contribute to the disappearance of ^{99m}Tc DTPA. Time-activity curves were obtained and half life values calculated by fitting a mono-exponential equation to the experimental data. A progressive reduction in ^{99m}Tc DTPA was recorded from the nasal and tracheobronchial airways and from the bronchoalveolar airway, suggesting that absorption was occurring. The disappearance of ^{99m}Tc DTPA was fastest from the bronchoalveolar region, which also had the largest mucosal surface. The similar shape of the retention curves for the nasal and tracheobronchial regions suggests that the characteristics of nasal absorption of ^{99m}Tc DTPA could prove applicable to the tracheobronchial region. It is proposed that the present methods are suited for comparing the pharmacology of small solute absorption across nasal, tracheobronchial, and bronchoalveolar airway mucosa.

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Clearance of technetium-99m labelled diethylenetriamine penta-acetate (^{99m}Tc DTPA; MW 492) has been used extensively to examine the integrity of the barriers in the bronchoalveolar region of the airways.[1 2] The tracer is usually administered as an aerosol, and its subsequent disappearance is monitored by external counting of the radioactivity over the chest. With this mode of administration the tracer is deposited, to a variable degree, in the conducting airways and in the alveoli. Most frequently an aerosol with a small particle size is used to favour alveolar deposition.[3] The tracer is cleared by the pulmonary rather than the bronchial circulation.[4] The rate of disappearance from the peripheral bronchoalveolar region is largely independent of blood flow[4] and the part cleared by the lymphatics is small.[5] Hence the clearance of ^{99m}Tc DTPA from the bronchoalveolar region of the airways mainly reflects the transfer of tracer across the alveolocapillary barriers.

A more proximal deposition of the tracer can be obtained if an aerosol with larger particle size is used, and attempts to do this have been made to determine clearance of ^{99m}Tc DTPA acoss the ciliated epithelium.[6–9] With the tracer administered as an aerosol it is difficult if not impossible to achieve deposition specifically restricted to the ciliated epithelium. Furthermore, it has been estimated that as much as two thirds of the tracer when applied as an aerosol to the human tracheobronchial mucosa might be cleared by mucociliary activity.[10] Tracheostomy and subsequent topical superfusion of the tracer has been carried out in animals,[11] but the effects of surgery and the role of mucociliary clearance on the total clearance of ^{99m}Tc DTPA in these experiments remain unknown.

In contrast to the limited accessibility of the tracheobronchial mucosa, the nasal mucosa can be reached with ease. Thus the nasal airways may provide a convenient model of the ciliated respiratory mucosa. Comparisons of clearance of ^{99m}Tc DTPA between the different airway regions are scarce and the available studies have given inconsistent results. Bhalla *et al*,[11] who superfused a solution of the tracer through a tracheostomy, reported that clearance of ^{99m}Tc DTPA was faster in the tracheobronchial region than in the bronchoalveolar region, the latter being exposed to aerosolised tracer. Oberdörster *et al*,[9] who gave inhalations of ^{99m}Tc DTPA to dogs, reported that alveolar absorption was faster than bronchial absorption. Bhalla *et al* [11] also reported that ^{99m}Tc DTPA was very poorly absorbed from the nasal mucosa. In these latter experiments surgery and dental impression cream were used to create a controlled distribution of the nasal instillate.

In the present study we have used non-traumatic techniques to study the absorption of ^{99m}Tc DTPA delivered selectively to guinea pig nasal, tracheobronchial, and bronchoalveolar regions.[12] We have used an external detection device to control the distribution of ^{99m}Tc DTPA and to make measurements of its rate of absorption.

Methods

STUDY DESIGN

The study was designed to determine the nasal (n = 6), tracheobronchial (n = 6), and bronchoalveolar (n = 6) clearance of ^{99m}Tc

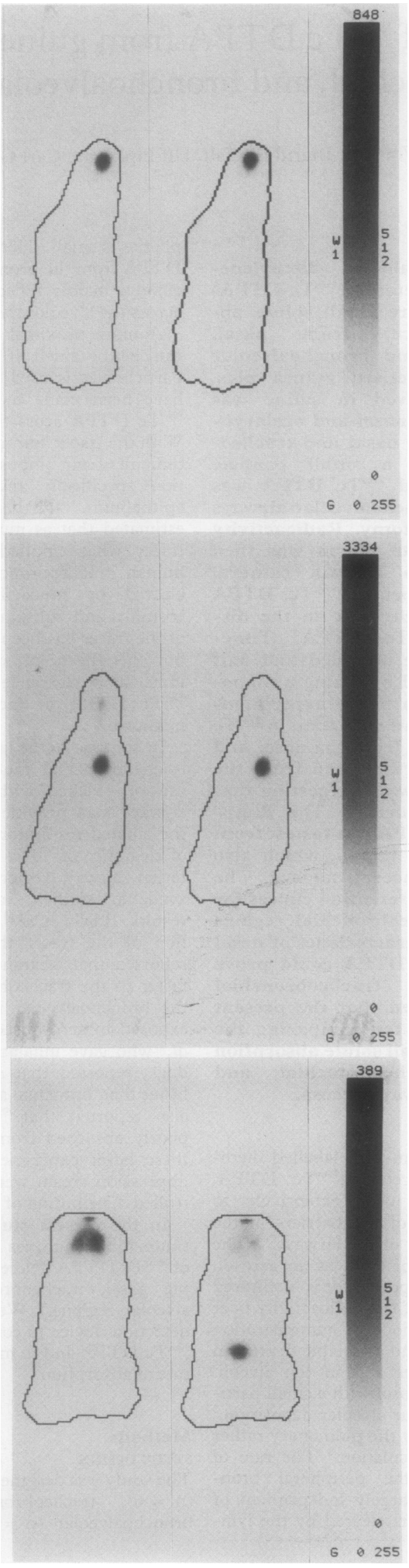

Figure 1 Distribution of ^{99m}Tc DTPA (dark areas) in the nasal (a), tracheobronchial (b), and bronchoalveolar (c) airways, immediately after instillation (left panels) and 20 minutes later (right panels). The contours show the guinea pig body (including the head in a and b but not in c). No redistribution of the margins of the boluses were detected during the course of the measurements. Bronchoalveolar deposition (c) was associated with substantial absorption of ^{99m}Tc DTPA, which was rapidly excreted into the urine (the urinary bladder is dark at the 20 minute recording—c right). Persistent activity was also detected in the region of the tracheostomy (c), possibly as a result of impaction of the aerosolised tracer.

DTPA in the guinea pig under normal conditions.

PREPARATION OF ANIMALS

Male guinea pigs weighing 400–900 g, after being starved for three to four hours were anaesthetised with a 3:2 mixture of ketamine (Ketalar 50 mg/ml) and xylazine (Rompun 20 mg/ml) given intramuscularly (1 ml/kg). These drugs maintain stable anaesthesia during the experiment and they cause little change in blood pressure.[13]

TRACER ADMINISTRATION

Nasal administration

A plastic tube (external diameter 0·61 mm) was introduced about 3 mm into one of the nasal cavities through the anterior nasal opening. The animals were placed in a supine position with the neck slightly extended and positioned under a gamma camera. Five millilitres of a solution containing ^{99m}Tc DTPA (Pentatate II, Amersham International, Amersham, UK) (about 2 MBq) and saline was infused at a constant rate over 15 seconds (Sage Servo pump).

Tracheobronchial administration

The animals were inclined to a 45° angle and a plastic tube (external diameter 0·61 mm) was atraumatically introduced into the tracheal lumen via the mouth.[14] After placing the animals (in the tilted position) under a gamma camera, 40 μl ^{99m}Tc DTPA (about 8 MBq) in saline was superfused on to the tracheal mucosa at a constant rate over two minutes.

Bronchoalveolar administration

A tracheostomy was performed and a plastic tube was introduced and secured with a ligature. Ventilation was supported with a Servo Ventilator 900C (Siemens Elema AB, Solna, Sweden). Ventilation was pressure controlled. A positive end expiratory pressure of 2·0 cm H_2O was applied and the mean (SEM) insufflation pressure was 12·6 (0·4) cm H_2O. The tidal volume was 8·0 (0·4) ml. The ^{99m}Tc DTPA solution (about 2 MBq) was nebulised with an air jet nebuliser (MA2, Viasol, Malmö, Sweden) and administered through the breathing circuit.[15] The particle size was 3·2 μm (mass median diameter) as measured with a laser light scattering technique.

CLEARANCE MEASUREMENTS

The distribution and the rate of disappearance of ^{99m}Tc DTPA were determined continuously for 20 minutes by means of a gamma camera (Portacamera, General Electric, Milwaukee, Wisconsin, USA) equipped with a converging collimator. Images were obtained in successive one minute frames and stored in a 64 × 64 image matrix. Regions of interest were selected over the nasal airways, the tracheobronchial airways, and the lung fields. Time-activity curves were generated from the regions and the half life values were calculated by fitting a monoexponential equation to the experimental data by the least squares method. The small solute absorption was expressed as the half life

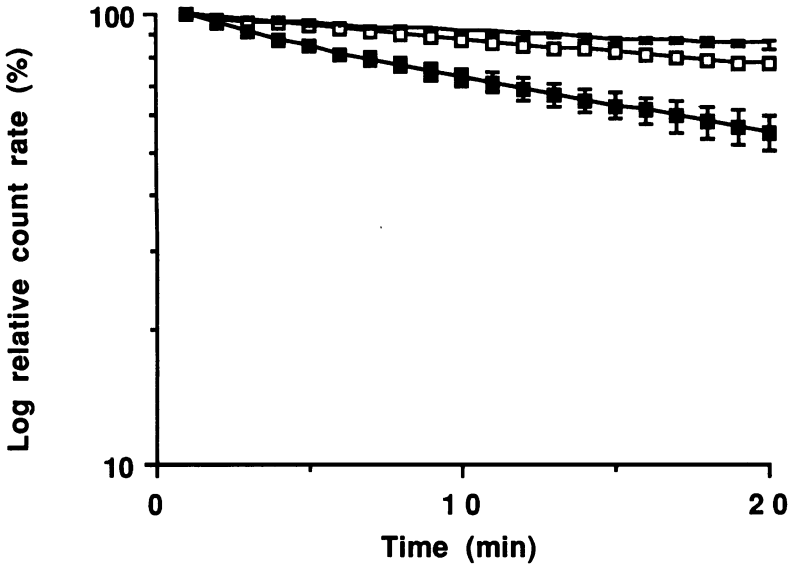

Figure 2 Time-activity curves (mean values with SEM) obtained from the nasal (upper line), tracheobronchial (open squares), and bronchoalveolar (filled squares) airways. The time-activity curves for the nasal, tracheobronchial, and bronchoalveolar airway regions are similar in shape but show with quantitative differences.

of the tracer in the airways. A region of interest was also selected immediately proximal to the tracheobronchial instillate to detect elimination of ^{99m}Tc DTPA from the test area by mucociliary activity.

ANALYSIS

The statistical evaluation was performed on a microcomputer (Macintosh, Apple Computer, Cupertino, California) with a software package (Statview 512+, Brainpower Inc, Calabasas, California). An analysis of variance was used to explore the differences in half life. If a significant value emerged, further analyses were made with Student's *t* test. P values less than 0·05 were considered significant (two tailed test).

Results

After instillation the radioactivity could be seen to form well defined and restricted boli in the nasal and tracheobronchial larger airways (fig 1*a*, 1*b*). With time the count rate over the instillates decreased, but there was essentially no visible change in the shape of the boli during the recording time of 20 minutes (fig 1*a*, 1*b*). In particular, no movement of tracer was detected by selecting a region of interest distal to the boli in the nasal airway or proximal to the bolus in the tracheal airways (fig 1*a*, 1*b*). After aerosol administration the distribution of radioactivity remained uniform throughout the lung fields (fig 1*c*). Persistent activity was detected in the region of the tracheostomy, possibly arising from impaction of the aerosolised tracer and disturbed blood flow in that region resulting from the applied ligature (fig 1*c*). In all groups a diffuse tissue background radioactivity gradually appeared, and at the end of the period of measurement radioactivity could be detected in the urinary bladder (fig 1*c*).

Time-activity curves generated from the regions selected over the nasal airways, tracheobronchial airways, and lung fields showed stable rates of disappearance of ^{99m}Tc DTPA (fig 2). The mean (SEM) half life values for nasal, tracheobronchial, and bronchoalveolar applications were 101 (13·9), 58·6 (4·5), and 24·8 (3·3) minutes ($p < 0·01$, analysis of variance). The differences in half life values between the nasal and tracheobronchial ($p < 0·05$), nasal and bronchoalveolar ($p < 0·01$), and tracheobronchial and bronchoalveolar ($p < 0·01$) applications were significant.

A time-activity curve generated from a region proximal to the tracheobronchial instillate, including the laryngeal, pharyngeal, and proximal oesophageal areas, also presented a retention curve. The mean (SEM) half life was 30·7 (6·2) minutes.

Discussion

Under normal conditions there are several barriers in the airways through which luminal molecules must pass before entering the circulation: the phospholipid-mucus liquid layer covering the airway mucosa, the airway mucosal or alveolar epithelial lining and its basement membrane, and the vascular endothelial lining and its basement membrane. This study examined the passage of ^{99m}Tc DTPA from the airway lumen into the circulation. We therefore studied the absorption permeability of the airway barriers for a small solute. Our data cannot be used to indicate permeability for large molecules, nor can they be used to indicate any exudation-transudation permeability of these barriers.[16]

The passage of ^{99m}Tc DTPA into the circulation causes a progressive increase in the contribution of background activity to the measured counts in the selected test area. Thus the decrease in counts in a given area is partially offset by an increase in background counts.[17] The effect would have been to underestimate the rate of disappearance from the bronchoalveolar region in particular, where the disappearance rate was high. The tracer is rapidly cleared from the circulation by glomerular filtration, however,[18] and in this study we used a rather brief period of scanning. Both these factors reduce the influence of background activity. We therefore expect this source of error to be small in the present study.

In the bronchoalveolar airways ^{99m}Tc DTPA diffuses passively across the airway barriers via an intercellular route before entering the circulation through the normal microvascular exchange of small solutes.[2] The evidence currently available suggests that the rate limiting barrier is the mucosal lining and its covering liquid layer.[2] The clearance rate, though relatively high under normal conditions, may be accelerated in conditions where these barriers are defective.[2,19] In the present study we observed that the clearance rates of ^{99m}Tc DTPA from the nasal, tracheobronchial, and bronchoalveolar mucosa were ranked according to surface area. Similarly, Oberdörster *et al*,[9] who studied aerosol administration in beagle dogs, observed a faster disappearance of ^{99m}Tc DTPA from the bronchoalveolar than from the tracheobronchial region. These data

are consistent with the possibility that ^{99m}Tc DTPA is cleared from the nasal and tracheobronchial regions in the same way as from the bronchoalveolar region—that is, through passive diffusion along concentration gradients across a rate limiting intercellular epithelial route.

When administered as an aerosol ^{99m}Tc DTPA is deposited on alveolar as well as ciliated epithelum. In the present study an aerosol with particles of 3·2 μm was used. In separate experiments, using an autoradiographic technique in rabbits, this particular aerosol has been shown to have the desired peripheral distribution (P Wollmer, unpublished observation). Possibly an aerosol of even smaller particles would increase the bronchoalveolar:tracheobronchial deposition ratio further and reduce the overlap between the regions. The distribution of radioactivity in this study (fig 1*c*), however, suggests that the bulk of the administered aerosol was deposited in the bronchoalveolar region.

Aerosols with larger particle size have been used in an effort to study the mucosal absorption permeability of the tracheobronchial epithelium specifically. The use of this technique is limited by the difficulties of obtaining defined and sufficient aerosol deposition and by the fact that deposition characteristics may be altered by properties induced by airway disease. Furthermore, recent studies by Bennett *et al*[10] have estimated that mucociliary transport activity makes a major contribution (about two thirds) to the total clearance of inhaled ^{99m}Tc DTPA from the tracheobronchial airways. In the present study these problems were circumvented. ^{99m}Tc DTPA was applied selectively to the tracheobronchial mucosa by superfusions via an orolaryngeal catheter. The very small volume of fluid introduced into the tracheobronchial airways with this technique is without effect on breathing and maintains the integrity of the mucosal barriers—that is, it does not increase the passage of plasma tracers into the airway lumen.[12] In agreement with previous observations,[12] the present superfusion technique distributed the tracer selectively to the lower part of the trachea and bronchi with some exposure of the small airways but without affecting the peripheral parts of the lung.

We observed no transport of the deposited ^{99m}Tc DTPA above the initial demarcation level in the trachea. In the tracheal area proximal to the selected tracheobronchial test area about a quarter of the activity was present immediately after instillation. The presence of ^{99m}Tc DTPA in this region may in part be caused by the retraction of the orolaryngeal catheter, which may move deposited tracer material upwards. Mucociliary transport would have decreased the disappearance rate from the area proximal to the selected tracheobronchial test area. As, however, the disappearance of ^{99m}Tc DTPA from the proximal area was faster than that from the test area mucociliary transport is unlikely to be making a large contribution to the clearance of the superfused tracer. The apparent lack of mucociliary escalator transport of ^{99m}Tc DTPA is not explained but may be due to a combination of several factors, including the administration of fluid, albeit a small volume. The anaesthetics and the inclined position of the animals may also have reduced the mucociliary transport rate. The unchanged distribution of radioactivity suggests that the tracheobronchial diffusion area for ^{99m}Tc DTPA was the same during the course of the measurements and that the rate of disappearance depended mainly on absorption processes.

The rate of absorption of ^{99m}Tc DTPA across the tracheobronchial mucosa was intermediate between the faster disappearance from the bronchoalveolar region and the slower disappearance from the nasal region, possibly reflecting the different sizes of mucosal surface areas from which ^{99m}Tc DTPA is absorbed. The different thicknesses of the airway barriers, in particular the epithelial lining, and variations in the phospholipid-mucus layers covering the mucosal surfaces may also play a part. More detailed comparisons of diffusion rates cannot be made as the exposed surface areas and the surface concentrations of ^{99m}Tc DTPA are not known.

Many findings in this study appear to be at variance with data reported by Bhalla *et al*.[11] These workers observed low rates of absorption of ^{99m}Tc DTPA from the nasal mucosa and higher rates of absorption from the trachea than from the bronchoalveolar region. The two studies were carried out with different techniques, however, a major difference being that nasal and tracheobronchial applications of ^{99m}Tc DTPA in our study were carried out by procedures causing minimal trauma, whereas Bhalla *et al* used surgery and other measures, which may have disturbed mucosal barrier functions and blood supply in both the nose and the trachea. The distribution of ^{99m}Tc DTPA was well controlled in both nasal and tracheobronchial airways by the present methods, indicating that the more complicated and traumatic techniques used by Bhalla *et al* may not be required.

In conclusion, the present study in guinea pigs provides data on the disappearance of ^{99m}Tc DTPA from nasal, tracheobronchial, and bronchoalveolar regions. The consistent absorption rates from these three parts of the airways indicate that the present methods are suited for further intervention studies and that the absorption of small solutes can be compared pharmacologically in the three sites.

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