The Role of the Bronchial Vasculature in Soluble Particle Clearance

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Although a role for the airway circulation in the clearance of inhaled particles is generally assumed, there is little information to confirm its importance. We studied the effects of decreased bronchial blood flow on the uptake of the soluble tracer technetium=99m–labeled diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) from subcarinal airways in sheep (n = 7). The bronchial artery was cannulated and perfused with autologous blood at a control flow (0.6 mL/min/kg) or when the perfusion pump was stopped (no flow). ^{99m}Tc-DTPA (6–10 µL) was delivered by a microspray nozzle inserted through a bronchoscope to a fourth-generation bronchus both during control blood flow conditions and no-flow conditions. Airway retention (by scintigraphy) and blood uptake were monitored for 30 min after the local deposition of ^{99m}Tc-DTPA. During control flow conditions, 30 min after the delivery of the radiolabel, 21% of the tracer remained at the deposition site. Of the total delivered tracer, maximum blood uptake was 18% (n = 3). When bronchial perfusion was stopped, airway retention 30 min after deposition increased to 43%, and maximum blood uptake decreased to 7% of the total delivered tracer. Although mucociliary clearance was not directly measured, radiolabel tracer was observed to move progressively from the deposition site up to larger airways and contributed to the overall removal of tracer from the site of deposition during both flow conditions. However, these results demonstrate that decreased bronchial perfusion increases airway retention by limiting vascular uptake of the soluble tracer. These results emphasize the importance of normal perfusion of the airway vasculature for uptake of therapeutic agents delivered specifically to the conducting airways. *Key words*: airway circulation, bronchial circulation, clearance, mucociliary transport, sheep, ^{99m}Tc-DTPA. — *Environ Health Perspect* 109(suppl 4):563–565 (2001). *http://ehpnet1.niehs.nih.gov/docs/2001/suppl-4/563-565wagner/abstract.html*

Although a role for the airway circulation in the clearance of inhaled particles is generally assumed, there is little information to confirm its importance (*1*,*2*). The subject has broad interest when considering particle dispersion for soluble species (therapeutic and harmful as well, e.g., antigenic) that deposit onto the delicate epithelial surfaces of the conducting airways. Recently, the importance of the circulation for the maintenance of normal mucociliary transport has been reported (*3*). Although the primary clearance pathways for insoluble and soluble particles differ (mucociliary transport vs intravascular absorption), it is likely that both rely heavily on the extent of airway perfusion. The bronchial artery perfuses the airways from the level of the carina to the terminal bronchioles, supplying the mucosal plexus as well as the parallel adventitial plexus located external to the airway smooth muscle (*4*). The mucosal capillary plexus, located in the subepithelial space, provides systemic access for all soluble substances that are deposited on the airway surface and that traverse the bronchial epithelium. Thus, selective alteration of the perfusion of mucosal capillaries underlying the epithelial basement membrane may affect the efficiency of particle uptake. Decreases in bronchial blood flow due to systemic hypotension, left atrial hypertension, increased airway pressure/lung volume, or lung transplantation could alter particle uptake of therapeutic as well as toxicologic aerosols deposited on the airway surface. Little experimental information is available regarding soluble particle uptake into the vasculature of the conducting airways. After infusing the soluble hydrophilic tracer technetium-99m–labeled diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) into an isolated sheep tracheal segment, Hanafi and colleagues reported the appearance of the tracer in a cannulated tracheal vein (*5*). Paradoxically, in their *in situ* preparation, tracer uptake into the circulation was shown to be inversely related to the level of tracheal blood flow. In the present study, we hypothesized that removal of soluble particles deposited onto an intrathoracic, subcarinal airway would be dependent on bronchial blood flow. We proposed that decreasing bronchial perfusion would decrease vascular absorption of a soluble tracer applied to the airway surface. We tested this hypothesis *in vivo* by limiting the blood supply to the airways and measuring the kinetics of soluble particle clearance.

Materials and Methods

The study protocol was approved by The Johns Hopkins Animal Care and Use Committee. Anesthesia was induced in sheep (25–35 kg) with intramuscular ketamine (30 mg/kg) and subsequently maintained with intravenous pentobarbital sodium (20 mg/kg/hr). The sheep were positioned supine on a surgical table, and after a tracheotomy was performed, the animals were intubated and paralyzed with pancuronium bromide (2 mg intravenously). The lungs were mechanically ventilated with a tidal volume of 10–12 mL/kg at a rate (12–15 breaths/min) sufficient to maintain normal blood gases. Positive end-expiratory pressure (5 cm H_2O) was applied. The left thorax was opened at the fifth intercostal space, and heparin (20,000 U intravenously) was administered. The esophageal and thoracic tracheal branches of the bronchoesophageal artery were ligated as previously described (*6*). The bronchial branch of the bronchoesophageal artery was isolated, cannulated, and perfused (0.6 mL/min/kg) with autologous blood withdrawn from the descending aorta and pumped through a variable-speed roller pump (*7*,*8*).

Local Tracer Delivery

The small (492 daltons), soluble, hydrophilic tracer ^{99m}Tc-DTPA was used to assess clearance from the airways and uptake by the bronchial circulation. DTPA was freshly prepared on each experiment day as ^{99m}Tc-labeled DTPA (Medi-Physics, Arlington Heights, IL, USA). Local airway delivery was performed to ensure deposition of the tracer exclusively onto surfaces of conducting airways perfused by the bronchial circulation. A fiberoptic bronchoscope (5 mm OD, Olympus Corp., Lake Success, NY, USA) was advanced through the trachea beyond the carina and into a fourth generation bronchus. A polyethylene catheter with a microspray nozzle at the tip was advanced through the working channel of the bronchoscope and visualized beyond the end of the bronchoscope (*9*). After momentarily stopping ventilation, 6–10 µL ^{99m}Tc-DTPA, which had been loaded into the catheter tip, was sprayed radially onto the airway wall. The catheter was then retracted into the channel of the bronchoscope, and the bronchoscope was removed from the animal.

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Controlled ventilation resumed, and serial dynamic images of clearance of the ^{99m}Tc-DTPA were acquired every 2 min for 30 min using NucLear MAC (Scientific Imaging, Inc., Littleton, CO, USA) and a gamma camera (MaxiCamera, General Electric, Waukesha, WI, USA). Radioisotope delivery and clearance data were quantitated with techniques modified from Foster and Stetkiewicz (*10*). The initial bronchial image acquired immediately after delivery of the ^{99m}Tc-DTPA was stored on a video screen and this enabled a region of interest to be selected by cursor manipulation and drawn to cover the site of ^{99m}Tc-DTPA delivery. For the clearance of ^{99m}Tc-DTPA, activity time plots were constructed for the region of interest. The retention of radioactivity during the 30-min washout was corrected for radioactive decay and expressed as a percentage of the ^{99m}Tc-DTPA delivered to the region at time 0 (immediately after the nozzle catheter and bronchoscope were withdrawn from the bronchial airway). The average retention time was calculated as $\sum$ activity × time/$\sum$ activity.

Blood Uptake

To determine blood uptake of tracer, we acquired systemic venous blood samples (0.5 mL) every 6 min for the 30-min period. Activity in blood was counted in a gamma counter (GammaTrac, TmAnalytic, Tampa, FL). To estimate the total amount of radiolabel in the blood of the sheep, we calculated the nominal blood volume equal to 8.5% body weight (*11*). We estimated total blood ^{99m}Tc-DTPA by multiplying counts per minute per milliliter by total blood volume; results are presented as a fraction of the total delivered ^{99m}Tc-DTPA.

Protocols

Airway clearance and blood uptake of ^{99m}Tc-DTPA were measured in sheep during control bronchial artery perfusion and when bronchial perfusion was stopped by turning off the perfusion pump. Blood flow was altered 5 min prior to the delivery of ^{99m}Tc-DTPA. To eliminate issues of attenuation of radioactivity due to regional differences in airway geometry and the subsequent two-dimensional image acquisition, we performed all evaluations (control vs no flow) as paired comparisons in the same airways. Thus, the experimental design of the protocol was to use each animal preparation as its own control, obtain measurements of ^{99m}Tc-DTPA, clearance during control flow conditions, followed by redelivery of ^{99m}Tc-DTPA and measure clearance during no-flow conditions. Bronchoscopy was performed by mapping into a specific fourth generation bronchus for the control flow deposition. The subsequent deposition during the interruption of bronchial blood flow took place in the same location.

Statistics

All data are presented as mean values ± standard errors. The Wilcoxon signed ranks test was used to determine differences between the two bronchial blood-flow conditions. $p < 0.05$ was accepted as significant.

Results

We studied seven sheep (29 ± 3 kg body weight) in which control bronchial blood flow averaged 18 ± 2 mL/min and resulted in a bronchial artery perfusion pressure of 86 ± 8 mmHg. Systemic arterial pressure averaged 93 ± 2 mmHg and peak inspiratory pressure was 17 ± 2 cm H_2O. Figure 1 shows a representative experiment, documenting the method for measuring ^{99m}Tc-DTPA clearance from the deposition site. Additionally, as the radioactive tracer was observed to move progressively up the airway tree (cranially), the series demonstrates that much of the label was removed from the deposition site by mucociliary clearance. This was a consistent observation among all the animals studied. The actual retained activity as a function of that originally deposited (100%) for three time points is shown in Figure 2. Within 30 min most of the ^{99m}Tc-DTPA was cleared from the deposition site. At 30 min an average of 21.1 ± 5.4% of the deposited label remained within the airway site. The average retention time for ^{99m}Tc-DTPA clearance was 10.5 ± 1.1 min. When the bronchial perfusion pump was turned off (no flow) prior to the second airway deposition, ^{99m}Tc-DTPA retention increased. The activity retained during no flow and at the same deposition site is also presented in Figure 2. Retained activity at 30 min was 42.6 ± 10.9% ($p = 0.03$ compared to control flow). Average retention time also increased to 13.3 ± 0.6 min ($p = 0.03$ compared to control flow). To further characterize the clearance pathways, bronchial vascular absorption was estimated by measuring the amount of radiolabel in systemic blood samples every 6 min throughout the 30-min time course. The total ^{99m}Tc-DTPA present in blood was extrapolated from the measured amount in a 0.5-mL venous blood sample and the total blood volume of the sheep [estimated as 8.5% of body mass in kilograms; (*11*)]. Thus, ^{99m}Tc-DTPA activity in the blood could be calculated as a fraction of the total delivered to the animal. A comparison of average blood activity during control flow and no-flow conditions is shown in Figure 3. The time to maximum blood activity was 12–18 min in the three animals during control flow conditions. The maximum values during control flow and their corresponding values at the

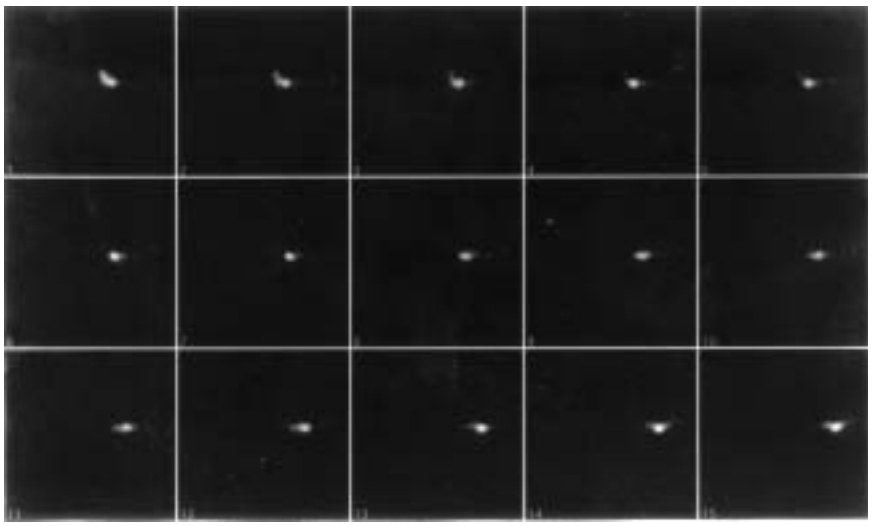

Figure 1. Serial gamma camera images of ^{99m}Tc-DTPA in airways in one sheep. ^{99m}Tc-DTPA image (white spot) deposited in a subcarinal airway appears to move to the right (cranially) over the course of 15 frames (30 min).

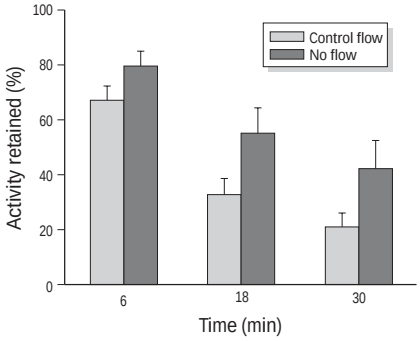

Figure 2. Time course of clearance of ^{99m}Tc-DTPA from subcarinal airway during control flow and when bronchial artery perfusion is interrupted (no flow). Average (± SE) radioactivity retained at the site of deposition in seven sheep at three time points.

same time during no-flow conditions are as follows: 18.4 versus 4.2%, 10.2 versus 6.8%, and 10.7 versus 6.6%. In each experiment ^{99m}Tc-DTPA activity absorbed into blood during no-flow conditions was considerably less than during control flow conditions. However, it should be noted that the maximum activity in the blood at any time point was always less than 20% of the delivered label. Furthermore, during control conditions approximately 80% of ^{99m}Tc-DTPA deposited onto the airway wall of a conducting airway was removed from the deposition site. During the no-flow conditions less than 10% was absorbed into the blood and approximately 60% was removed from the deposition site. Thus, a significant fraction of the ^{99m}Tc-DTPA likely was cleared by mucociliary function, as observed in Figure 1.

Discussion

The results of this study in anesthetized sheep demonstrate the importance of the bronchial circulation in the clearance of small, soluble substances deposited onto the subcarinal airways. Interrupting bronchial artery perfusion resulted in both an increased retention of soluble tracer at the site of deposition and an attenuation in the uptake of ^{99m}Tc-DTPA into the systemic blood. Additionally, during both flow conditions, much of the delivered tracer could not be accounted for by the combined activity at the site of deposition and the maximum levels in the blood. Although clearance from the blood by the kidneys might contribute, the amount of label that needed to be accounted for was too high to be removed exclusively by excretion (*12*). Furthermore, serial images of the conducting airways demonstrated that a significant fraction of the deposited label appeared to move by mucociliary action. Thus, the two major observations of this series of experiments were the bronchial blood flow dependence of soluble tracer kinetics and mucociliary clearance

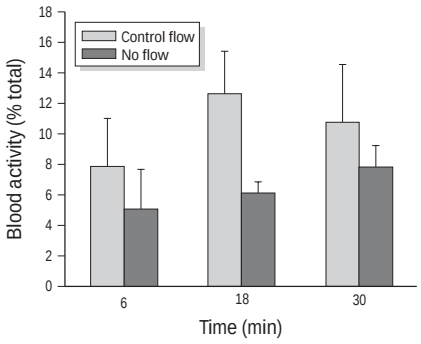

Figure 3. Average (± SE) ^{99m}Tc-DTPA activity in blood as percent of total delivered to the airway at three time points. *n* = 3 sheep.

of a soluble substance deposited in the conducting airways.

The mechanisms responsible for the blood flow dependence of soluble tracer kinetics are related to both a decrease in the blood uptake of the soluble tracer and a presumed decrease in mucociliary clearance of the tracer. Blood uptake was only attenuated and not completely absent when blood flow was interrupted. This result is likely related to two factors. First, interruption of perfusion does not prevent collateral flow at downstream sites. Baile et al., using aggressive methods to obliterate the bronchial vasculature, demonstrated a persistent, albeit greatly decreased, perfusion to the precapillary vessels, even after occluding the main bronchial arterial inflow in sheep (*13*). Additionally, as mucociliary activity was present during both flow conditions, delivered ^{99m}Tc-DTPA did not remain exclusively at the site of deposition. At later time points when tracer was in transit through the tracheal airway, uptake by blood vessels in the trachea where blood flow was not controlled likely contributed to the total systemic venous ^{99m}Tc-DTPA activity. Thus examination of the blood activity during early time points more accurately reflects blood uptake in the subcarinal airways before the mucociliary transport of surface activity into the tracheal airway.

We have previously reported the dependence of mucociliary clearance on bronchial perfusion (*3*). Decreased bronchial artery perfusion resulted in a significant slowing of the mucociliary clearance of the inert, insoluble tracer, ^{99m}Tc-sulfur colloid deposited in the conducting airways. We speculated that the mechanism for the reduction in particle clearance relates to the changes in cells or structures dependent on normal bronchial blood flow. Changes in nutrient flow, wall temperature and/or humidity, shear-induced mediator release, or ischemic injury might all account for this observation. Thus, in the present study it is presumed that impaired mucociliary function as well as decreased blood uptake contributed to the increased retention of ^{99m}Tc- DTPA at the deposition site.

Our results, however, are inconsistent with previous observations reported by Hanafi et al., who examined soluble tracer kinetics in an isolated tracheal segment (*5*). Using an *in vivo* sheep preparation, these investigators filled an isolated tracheal segment with ^{99m}Tc-DTPA in a physiologic salt solution and then measured tracheal artery and venous blood flow. Thus, the experimental preparation eliminated the mucociliary component as a potential pathway of clearance. These investigators showed that decreasing perfusion resulted in an increase in the venous uptake and clearance of the tracer. They suggested that decreased perfusion resulted in a decrease in interstitial fluid volume of the airway wall, which might decrease the barrier to tracer uptake into the vasculature. Another potential explanation provided was related to the fact that in their experimental setup, arterial inflow was never equal to the venous outflow of the isolated segment. Thus, perfusion redistribution through collateral vascular channels might have been altered with changes in inflow, obscuring the true changes in tracer concentrations.

In summary, as a first step toward defining the preferential clearance pathways of soluble particles deposited onto intrathoracic airways, we have shown *in vivo* that bronchial blood flow is essential for absorption and redistribution of the particles, and their clearance along the airway surface. Aqueous particles were removed from the subcarinal surface by two dynamic processes: *a*) dispersion into the subepithelial vascular network lining the airways and transferred to the systemic blood pool, and *b*) transport along with respiratory mucus by mucociliary clearance into the tracheal airway. The effectiveness of both of these processes is impaired when bronchial perfusion becomes limited.

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