Importance of airway blood flow on particle clearance from the lung

ELIZABETH M. WAGNER AND W. MICHAEL FOSTER
Departments of Medicine and Environmental Health Sciences, The Johns Hopkins University, Baltimore, Maryland 21224

Wagner, Elizabeth M., and W. Michael Foster. Importance of airway blood flow on particle clearance from the lung. J. Appl. Physiol. 81(5): 1878–1883, 1996.—The role of the airway circulation in supporting mucociliary function has been essentially unstudied. We evaluated the airway clearance of inert, insoluble particles in anesthetized ventilated sheep (n = 8), in which bronchial perfusion was controlled, to determine whether airway mucosal blood flow is essential for maintaining surface transport of particles through airways. The bronchial branch of the bronchoesophageal artery was cannulated and perfused with autologous blood at control flow (0.6 ml·min⁻¹·kg⁻¹) or perfusion was stopped. With the sheep in a supine position and after a steady-state ¹³¹I-Xe ventilation scan for designation of lung zones of interest, an inert ⁹⁹mTc-labeled sulfur colloid aerosol (2.1-μm diameter) was deposited in the lung. The clearance kinetics of the radiolabeled particles were determined from the activity-time data obtained for right and left lung zones. At 60 min postdeposition of aerosol, average airway particle retention for control bronchial flow conditions was 57 ± 7 (SE)% for the right and 53 ± 8% for the left lung zones. Clearance of particles was significantly impaired when bronchial blood flow was stopped, e.g., right and left lung zones averaged 77 ± 6 and 76 ± 7% at 60 min, respectively (P < 0.05). These data demonstrate a significant influence of the bronchial circulation on mucociliary transport of insoluble particles. Potential mechanisms that may account for these results include the importance of the bronchial circulation for nutrient flow, maintenance of airway wall temperature and humidity, and release of mediators and sequela associated with tissue ischemia.

METHODS

Experimental preparation. Anesthesia of healthy sheep (23–35 kg) of mixed breeds was induced with ketamine (1 g im) and maintained throughout the surgical and experimental periods with pentobarbital sodium (15 mg/kg loading dose and 20 mg/kg hourly dose). Sheep were studied in the supine position and were paralyzed with pancuronium bromide (2 mg iv). A tracheostomy was performed with the tracheal cannula placed upstream of the zones of the lung that would be analyzed for mucociliary clearance. Sheep were mechanically ventilated at a tidal volume (12 ml/kg) and rate (12–15 breaths/min) sufficient to maintain blood gases within the normal range. Supplemental oxygen was provided, and, after thoracotomy, all sheep were placed on 5 cmH₂O positive end-expiratory pressure. Airway pressure and expired CO₂ were measured at a side arm of the tracheal cannula. Polyethylene catheters were inserted into the femoral vein for anesthetic infusion, into the femoral artery for systemic arterial pressure measurement, and into the other femoral artery to supply the pump used to perfuse the bronchial artery. A left lateral thoracotomy was performed between the fifth and sixth ribs to allow direct access to the bronchoesophageal artery as it exits the aorta. The thoracic tracheal and esophageal branches were ligated. After administering the anticoagulant heparin (20,000 USP units), the artery was cannulated with an 18-gauge catheter inserted directly into the vessel. After the catheter was secured in place, the bronchial branch of the bronchoesophageal artery was perfused with autologous blood from the femoral artery at a flow controlled by a calibrated roller pump and set initially at the control flow of 0.6 ml·min⁻¹·kg⁻¹, a value within the reported normal range for sheep (13). Bronchial artery pressure was measured at a side port of the inflow line. During the experimental protocol, just before the period of no bronchial flow, the bronchial cannula was gently flushed with heparinized saline. The infusion line was clamped close to the bronchial catheter, and the perfusion circuit was flushed with heparinized saline. Hemodynamic pressures were measured with Gould transducers, and airway pressure was measured with a Validyne transducer. Measurements were recorded on a Grass recorder. A 30-min stabilization period followed surgery.

Particle delivery and clearance measurements. The technique to measure the distribution of insoluble radioactive...
particles deposited in the lung and their subsequent clearance as a gauge of airway mucociliary transport by gamma-camera imaging has been described previously (17). 99mTc-labeled sulfur colloid particles were generated as an aerosol by jet nebulizer and delivered through the tracheal tube during tidal breathing. Before nebulization, prelabeled sulfur colloid was dialyzed with distilled water for a 5-min period to remove any free unbound Tc. To verify the labeling procedures, 99mTc-labeled sulfur colloid was sampled from the nebulizer reservoir postnebulization to assay for unbound 99mTc by using silica gel media and thin-layer chromatography (6). This radiomarker has been shown to be stable throughout a 24-h postinhalation period in human subjects (17). To standardize the delivery and dose of aerosols, a dosimeter (Spira-Elektra-2; Respiratory Care Center, Hameenlinna, Finland), which delivered aerosol only during the inspiratory cycle of a tidal breath, was attached to the nebulizer (Devilbiss no. 646, Somerset, PA), which was energized with filtered air at 30 pounds/inch². The aerosol output from the nebulizer was polydisperse in size (geometric SD = 2.8), with a mean mass aerodynamic diameter of 2.1 µm (determined by cascade impaction). The aerosol was delivered over five tidal breaths, with the nebulizer programmed to actuate over approximately the last 40% of the inspiratory phase, followed by a 5-s pause at end inspiration and two nonaerosol breaths before the next breath for which aerosol was delivered. The mean inspiratory flow rate was controlled by the ventilator. By using this pattern of aerosol delivery, regional lung counts were made. Images of radioactivity within the thorax were acquired with an anteriorly positioned Anger camera, set with a 15% window around the peak energy of 140 keV and shielded by a parallel hole collimator. Particle deposition was monitored during aerosol inhalation to achieve regional count rates of 5,000–7,000 counts/min above background. After inhalation of aerosol, serial images of the distribution of radioactivity were recorded.

To define a region of interest, a steady-state scan during ventilation with radiolabeled xenon gas (133Xe) was acquired immediately after the surgical recovery period and before radioaerosol inhalation (16). This scan aided in the proper alignment of the camera and counting field over the sheep based on the Xe ventilation image (see METHODS) have been drawn in and are represented on the lung image. In two sheep, Xe ventilation scans were also acquired at the end of the aerosol clearance period, and these ventilation scans were nearly identical to the initial scans with steady-state Xe counts for the entire lung field (initial 33,700 vs. final 32,300 counts) with regional distributions being approximately equivalent. This result suggests that regional ventilation was not markedly altered during the course of the experimental protocol. In the eight sheep evaluated, the right and left zones of the ventilation images contained on average 33 ± 2 and 39 ± 1% of the Xe counts, respectively, with the remaining counts contained in the third zone of the lung.

### RESULTS

Eight sheep weighing 30.7 ± 1.4 kg were studied. Mean arterial pressure at the start of the experiment was 97 ± 3 mmHg, and bronchial artery inflow pressure averaged 106 ± 6 mmHg at control bronchial blood flow (18.4 ± 0.9 ml/min). Peak inspiratory pressure was 16 ± 1 cmH₂O at the set tidal volume of 304 ± 17 ml. The lung image of a representative ventilation scan acquired in one of the sheep during steady-state breathing of Xe before aerosol challenge is shown in Fig. 1 (left). The designated lung zones of interest used for analysis of deposition and clearance of aerosol and based on the Xe ventilation image (see METHODS) have been drawn in and are represented on the lung image. In two sheep, Xe ventilation scans were also acquired at the end of the aerosol clearance period, and these ventilation scans were nearly identical to the initial scans with steady-state Xe counts for the entire lung field (initial 33,700 vs. final 32,300 counts) with regional distributions being approximately equivalent. This result suggests that regional ventilation was not markedly altered during the course of the experimental protocol. In the eight sheep evaluated, the right and left zones of the ventilation images contained on average 33 ± 2 and 39 ± 1% of the Xe counts, respectively, with the remaining counts contained in the third zone of the lung.

#### Table 1. Summary of studied animals

<table>
<thead>
<tr>
<th>No. of Sheep</th>
<th>Aerosol Clearance no. 1</th>
<th>Aerosol Clearance no. 2</th>
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<tr>
<td>2</td>
<td>60 min–Control flow</td>
<td>60 min–No flow</td>
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<td>2</td>
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<td>1</td>
<td>60 min–Control flow</td>
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<td>3</td>
<td>60 min–No flow</td>
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A representative lung image acquired after aerosol challenge is presented in Fig. 1 on right (same animal as imaged with ventilation scan on left). In the eight sheep imaged immediately after aerosolization and deposition of the Tc-labeled aerosol, zone deposition as a percent of the total aerosol lung counts averaged 29 ± 1 and 39 ± 1% for the right and left zones, respectively. The average retention of aerosol counts (after background and decay correction) for each bronchial blood flow condition (control vs. no flow) is presented in Fig. 2 for the right (top) and left (bottom) lung zones. Differences in the percentage of retained aerosol particles for the two blood flow conditions were statistically significant (P < 0.05) for both lung zones at the respective 20, 40, and 60 min time points. During control bronchial blood flow, clearance of the aerosol particles appeared to have reached an asymptote at ~60 min postdeposition of aerosol. Aerosol retentions of the right and left lung zones at this time were 57 ± 7 and 53 ± 8%, respectively. Aerosol retention was significantly greater at this 60-min time point for the no-blood-flow condition and averaged 77 ± 6 and 76 ± 7% for the right and left lung zones (P = 0.0442 and 0.0384, respectively).

Although monitoring of aerosol retention was extended beyond the 60-min time point in most experiments, additional aerosol clearance was not observed for either lung zone or bronchial blood flow condition (P > 0.05). Further analysis of aerosol clearance data was performed by determining the area under the curve of retention vs. time plots for each aerosol challenge. This value provides an average retention time for particles. The average data calculated from five aerosol challenges for each blood flow condition are presented in Fig. 3. The average retention time for the condition of no bronchial blood flow was significantly greater for both the right (P = 0.0193) and left (P = 0.0190) lung regions, compared with the average retention time for control flow conditions. This represented delays in aerosol clearance of 19 and 24% from clearance established during control flow conditions for the right and left lung zones, respectively.

In two sheep, a second aerosol challenge was performed during conditions of control bronchial blood flow. The differences in the percent retention between the second and the first challenge are presented in Fig. 4. Each point represents aerosol retention (average of right and left lung zones) of the second challenge minus the first challenge at the specified time. For one of these sheep, differences between the two challenges were minimal at any time point, and for the other sheep, a slightly faster clearance was observed after the second aerosol challenge.

**DISCUSSION**

The influence of the airway blood supply on mucociliary function is poorly understood. It is generally assumed that the bronchial circulation provides flow of nutrient materials to cellular structures of the airway wall; however, the consequences of limiting airway perfusion have received little attention. In this study, we have demonstrated that when bronchial blood flow...
is stopped, there is a significant attenuation of mucociliary clearance of small insoluble aerosol particles. When the blood flow through the cannulated bronchial artery was stopped for a 1-h period, the transport of aerosol particles decreased. Impairment of clearance function was evident as early as 20 min after bronchial perfusion had been stopped, and after 1 h this represented, on average, a 50% reduction in clearance compared with control blood flow conditions.

The importance of bronchial perfusion for the clearance capacity of airways is emphasized when consideration is given to the percent of the sheep lung perfused by the bronchial artery. It has been estimated that the bronchoesophageal artery perfuses 60–90% of the intraparenchymal airways (1). Baile et al. (5) demonstrated, using radiolabeled microspheres, that ligation of the bronchoesophageal artery in sheep only attenuated regional flow by 50%. Although microspheres have been shown to have a significant vasodilating effect on this vascular bed (25), it is possible that at least a small amount of collateral flow from other systemic arteries could partially supply the airway wall under the conditions of an occluded bronchoesophageal artery. Thus, in our protocols, when we stopped bronchial blood flow, it is unlikely that perfusion was withheld completely from the airway tissues; and yet, we still observed a substantial effect on mucociliary function as assessed by airway clearance of insoluble particles.

The mechanism(s) responsible for this dependency of mucociliary clearance on bronchial blood flow can only be speculated on at this point. Several potential factors include the following.

1. Bronchial blood flow may provide essential nutrients to cells involved in mucociliary transport. The importance of substrate delivery to and metabolite removal from the variety of cells within the airway by the bronchial circulation is not certain. In extirpated tissues that are warmed and humidified, ciliary beating and transport of particles by airway epithelium can be sustained for several hours, although

Fig. 2. Lung retention of insoluble radiolabeled aerosol particles vs. time curves. Data presented are average retentions (±SE) for right (top) and left (bottom) lung zones observed in 5 sheep at indicated times for each condition: control bronchial blood flow (C) and no bronchial blood flow (○). Retention is expressed as percent of aerosol initially deposited in the lung zone during inhalation; t = 0 immediately follows inhalation of aerosol. *Significantly different from control bronchial blood flow condition (P < 0.05).

Fig. 3. Average retention time of aerosol particles in right and left lung zones. Mean aerosol retention times are compared for control and no-flow conditions (n = 5 for each condition); average retention time calculated from area under retention-time curve from t = 0 to 60 min postdeposition of aerosol. *Significantly different from control bronchial blood flow condition (P < 0.05).

Fig. 4. Reproducibility of aerosol clearance under conditions of control blood flow. For repeat studies of particle clearance, differences (lung retention of 2nd study minus retention of 1st study) in lung retention are indicated for times postdeposition of aerosol. Each line connecting • is for a single sheep; no difference between aerosol retentions is represented by solid horizontal line passing through ordinate at 0% retention.
an adequate mucus supply is required to preserve particle clearance (26). Perfusion may influence tonic neurotransmitter release, which has been shown to have significant modulating influence on mucociliary function (15, 17, 28, 31). 2) The airway circulation may play an important role in maintaining temperature of the airway wall. Studies examining the effects of cooling on the airway vasculature have shown a marked vasodilation when the airways are exposed to cold, dry air (3, 23). These observations have lead to the theory that the airway circulation dilates to maintain airway wall temperature and humidity in the face of decreasing airstream temperatures. Although we did not assess local tissue temperature gradients in our preparation during the period of no bronchial blood flow, a possible indirect effect on the mucosa and ciliated epithelium would be a decrease in temperature. Ciliary activity of airway tissues increases with increased temperature; the exact amount depending on whether temperature is measured locally or is the temperature of the ambient air (2, 7, 22). In our experimental condition, a decrease in mucosal temperature during cessation of bronchial blood flow may have reduced tissue temperature with corresponding reductions in ciliary beating and mucus transport. 3) Additionally, our observations may be related to the ability of the bronchial circulation to hydrate the airway, influence the composition of periciliary fluid, and indirectly alter the efficiency of ciliary beating, and reduce particle transport. Hirsch and colleagues (19) showed impairment of tracheal mucus velocity in dogs exposed to dry air. Finally, stopping perfusion to this vascular bed may lead to ischemic injury of the perfused tissue and result in the release of metabolites or mediators that are toxic to normal mucociliary function. This possibility was suggested by exploratory studies in which we found that normal clearance function recovered slowly after prolonged no-flow conditions. In fact, these early observations were influential to our study design, in that we elected not to perform any control clearance studies that were preceded by experimental periods of no bronchial blood flow.

The maximum amount of clearance averaged \(-50\%\) and was a somewhat unexpected finding. We assume that this was likely due to the deposition of aerosol in peripheral regions of the sheep lung where clearance mechanisms are considerably slower (21) and despite our attempts to limit penetration by only delivering aerosol during the final 40% of the inspiratory breath. Additionally, some of the particles may not have been available for clearance because they had penetrated the mucus layer or were deposited onto airway surfaces devoid of mucus and became retained at the surface of the epithelial cell membrane (27). If this phenomenon occurred more readily in the absence of bronchial perfusion, it would suggest a potential role for the bronchial circulation in maintenance of the fluid lining of the airways.

Several technical aspects regarding our experimental procedures require comment. We are convinced that unbound radiolabel did not leach from the colloid particles to be removed through a vascular route. Although we did not routinely sample the blood, on the occasions that we did, we were unable to detect any radioactivity in the blood samples. Furthermore, we know from previous work that the Tc label does not leach off of sulfur colloid particles and, therefore, is not available for clearance by the blood (6). As an added precaution during the preparation of the radiopharmaceutical, the final solution was dialyzed with distilled water for 5 min to remove any unbound radiolabel from the solution before aerosolization. Groth et al. (17) have used this radiomarker previously in human studies of mucociliary function and have satisfied any concerns regarding the stability of the labeled particles throughout a 24-h period deposition.

An additional concern was whether mucociliary clearance measured in this anesthetized sheep preparation would be reproducible over time. In two sheep, we administered two consecutive challenges and monitored clearance without manipulating bronchial blood flow. Particle clearance kinetics were approximately equivalent for the two challenges in each sheep (Fig. 4). This result justified our experimental design to perform two challenges and confirmed that the decrease in particle clearance observed with the no-flow condition was not a consequence of experimental time.

Although the effect of bronchial blood flow on bronchial mucociliary clearance has not been studied previously by direct assessment, the lung transplantation literature (experimental and clinical) provides some insight into the effects of the bronchial circulation on mucociliary clearance. A number of studies in animal models of allo- and autotransplantation of lung lobes have demonstrated that in the immediate postoperative period (a few days to several weeks), mucociliary clearance is impaired (8, 24). Initially, this may be due to a combination of factors, for example local obstruction at the site of bronchial anastomosis, which resolves within several days, and a longer term impairment of clearance that results from bronchial denervation and devascularization. Denervation is not thought to be the major pathogenic factor because of the relatively rapid rate of recovery during the period of persistent denervation. Denervation is believed to impact essentially on submucosal glands, which atrophy in the period after bronchial transection (8). Several studies have suggested that the bronchial vascular and lymphatic supplies of reimplanted lungs reestablish at \(1\) mo postimplant (4, 14, 30), although Baile et al. (4) have shown blood flow to exceed control levels by \(1\) wk posttransplantation. If during resection of the bronchus in dogs care is taken to preserve an associated flap of peribronchial tissue, then mucus transport abnormalities are prevented (24). In humans, however, reversal appears to be complicated by additional factors such as immune-suppression therapy, and, at least for studies of long-term survivors (1.5 yr posttransplantation) of heart and/or lung or double-lung transplantation, bronchial mucus clearance is suppressed compared with normal subjects (18).
In summary, we have demonstrated that when perfusion of the bronchial artery is stopped, mucociliary clearance of insoluble particles is significantly attenuated. Because of the potential for airway injury from retained toxic materials, it is important to extend these studies and evaluate the mechanisms by which airway perfusion modulates particle clearance.

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Address for reprint requests: E. M. Wagner, The Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Baltimore, MD 21224.

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REFERENCES


