Ligation of the bronchial artery in sheep attenuates early pulmonary changes following exposure to smoke

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Efimova, Olga, A. B. Volokhov, Sakineh Iliaifar, and C. A. Hales. Ligation of the bronchial artery in sheep attenuates early pulmonary changes following exposure to smoke. J. Appl. Physiol. 88: 888–893, 2000.—Smoke inhalation can produce acute pulmonary edema. Previous studies have shown that the bronchial arteries are important in acute pulmonary edema occurring after inhalation of a synthetic smoke containing acrolein, a common smoke toxin. We hypothesized that inhalation of smoke from burning cotton, known to contain acrolein, would produce in sheep acute pulmonary edema that was mediated by the bronchial circulation. We reasoned that occluding the bronchial arteries would eliminate smoke-induced pulmonary edema, whereas occlusion of the pulmonary artery would not. Smoke inhalation increased lung lymph flow from baseline from 2.4 ± 0.7 to 5.6 ± 1.2 ml/0.5 h at 30 min (P < 0.05) to 9.1 ± 1 ml/0.5 h at 4 h (P < 0.05). Bronchial artery ligation diminished and delayed the rise in lymph flow with baseline at 2.8 ± 0.7 ml/0.5 h rising to 3.1 ± 0.8 ml/0.5 h at 30 min to 6.5 ± 1.5 ml/0.5 h at 240 min (P < 0.05). Wet-to-dry ratio was 4.1 ± 0.2 in control, 5.1 ± 0.3 in smoke inhalation (P < 0.05), and 4.4 ± 0.4 in bronchial artery ligation plus smoke-inhalation group. Smoke inhalation after occlusion of the right pulmonary artery resulted in a wet-to-dry ratio after 4 h in the right lung of 5.5 ± 0.8 (P < 0.05 vs. control) and in the left nonoccluded lung of 5.01 ± 0.7 (P < 0.05). Thus the bronchial arteries may be major contributors to acute pulmonary and airway edema following smoke inhalation because the edema occurs in the lung with the pulmonary artery occluded but not in the lungs with bronchial arteries ligated.

Bronchial circulation; cotton smoke; lung lymph

Inhalation of smoke can result in severe respiratory complications, which are the leading cause of morbidity and mortality in fire victims (22, 26). Pulmonary edema is one of those complications and can occur acutely within 1–4 h, depending on the quantity of inhaled smoke toxin, such as acrolein (12), or may take a much more delayed course, presenting as acute respiratory distress syndrome at 24–48 h (3). Animal models of smoke inhalation have shown that the earliest changes following smoke exposure are an increase in bronchial blood flow followed by an increase in lung lymph flow and lymph content of protein with subsequent pulmonary edema (2, 11–15, 21). Histological studies have shown that the airways are swollen and edematous, but there is also peribronchial cuffing, septal thickening, and peribronchial alveolar edema, especially around the respiratory bronchioles (11–13, 15, 16, 18). The peribronchial cuffing may be due to the suspected negative pressure in this area acting as a sump (25). However, the peribronchial location of the edema may represent spillover from the edematous airways, which are supplied by the bronchial arteries.

Although the largest blood supply to the lungs from the pulmonary arteries, it has been demonstrated that the bronchial arteries, which account for only 1–3% of cardiac output at rest, can play a significant role in several pathological processes (4, 7–10). Wagner et al. (27) have shown that either an increase in bronchial artery flow or an increase in bronchial artery permeability can considerably increase lymph flow from the lung. Pietra et al. (20) used Pelican black as a tracer molecule to show increased permeability of peribronchial vessels after histamine infusion. We showed with another tracer molecule, Monastral blue B, that the peribronchial, not the pulmonary, vessels were the site of injury induced by inhalation of synthetic smoke containing acrolein and a carbon carrier (11). Surgical interruption of the bronchial arteries in dogs decreased pulmonary edema following exposure to synthetic smoke with acrolein (11). Continuous 24-h measurements of bronchial blood flow in sheep have shown a rapid and marked increase by 30 min after inhalation of smoke from burning cotton, which preceded the increase in lung lymph flow by several hours (1). Despite the disparity between bronchial blood flow and lymph flow onset, occlusion of the bronchial artery in the sheep resulted in a reduction in injury 24 h after smoke inhalation as assessed by gravimetric measurement (2). Sakurai et al. (23) have subsequently shown that both ligation and ethanol ablation of the bronchoesophageal artery reduce lung water after smoke inhalation, although more effectively in the right than left lung. They again noted an 8-h lag from smoke inhalation to the increase in lymph flow. The more rapid early rise in bronchial blood flow than in lung lymph flow has led to the suggestion that the bronchial arteries are not the direct source of the pulmonary edema but might be carrying smoke-released edematogenic mediators from the injured central airways through known anastomotic channels to the pulmonary circulation, which is the late source of edema (2). Sakurai et al. (23) have shown that, after inhalation of smoke from burning...
cotton, the microvascular reflection coefficient is reduced in the pulmonary vasculature; therefore, there is evidence to suggest they may leak. We wondered, however, if the failure to find an early increase in lung lymph flow after inhalation of smoke from burning cotton was methodological, as our laboratory has previously found an early rise in pulmonary lymph flow after inhalation of synthetic smoke with acrolein (11, 12). We, therefore, examined the time course of the increase in lung lymph flow after inhalation of smoke from burning cotton to see whether lung lymph flow rose early, in keeping with the known early rise in bronchial artery flow. We found an early rise in lung lymph flow and, therefore, examined the impact of bronchial artery ligation on this early rise in lymph flow and the ensuing pulmonary and airway edema. In addition, we ligated the right pulmonary artery in some sheep to see whether smoke inhalation could occur in the absence of the pulmonary artery flow, thus suggesting that the bronchial arteries could be a major source of the acute pulmonary edema seen after smoke inhalation.

METHODS

Animal Preparation

Eighteen sheep weighing 29–31 kg were anesthetized with intravenous thiopental sodium (25 mg/kg induction, 150- to 200-mg maintenance doses given intermittently to maintain deep anesthesia), intubated with a cuffed endotracheal tube (7.5 mm ID, 33 cm long), and ventilated (Harvard Apparatus, Millis, MA) with an initial setting of 0.50 inspired O2 fraction, 15 mg·ml⁻¹·kg⁻¹ tidal volume, 15 breaths/min, and 2-Torr positive end-expiratory pressure. The respiratory rate was adjusted to maintain arterial PCO₂ between 36 and 44 Torr. Blood gases and pH were measured at 38°C with an Instrumental Laboratory 1306 blood analyzer (Watertown, MA). An oral gastric tube was inserted to evacuate gastric contents. A catheter was inserted into a femoral vein to permit infusion of lactated Ringer solution at a rate sufficient to maintain a blood pressure of approximately 100 mmHg. A Swan-Ganz pulmonary artery catheter (model 93A-13 H-7F, American Edwards Laboratories, Santa Ana, CA) was inserted via the inferior vena cava into the pulmonary artery. Cardiac output was determined by the thermodilution technique (model PR 2, Instrument, Baltimore, MD). The remaining supernatants were frozen at -80°C for future assays.

Smoke Generation and Administration

Smoke was generated by burning cotton in a modified bee smoker (The Bee Keeper, Woburn, MA) as originally described by Walker et al. (28) and subsequently modified by Kimura et al. (16). The smoke was attached to the tracheal tube while four sets of 30 breaths were delivered. A total of 120 breaths of cotton smoke with a tidal volume of 15 mg/kg was given to each animal in the three groups. Temperature of the smoke was 39°C when it reached the endotracheal tube. In the intervals between smoke administrations, sheep were mechanically ventilated.

Statistics

All values were calculated as means ± SE. Data were compared by ANOVA for repeated measurements within groups, with P < 0.05 regarded as the significant difference (StatView 512+ statistical program; Brainpower, Calabasas, CA). All groups at baseline were also tested against each other by a factorial ANOVA to be certain that control values
were similar. If indicated, Scheffe’s test was used to identify significant groups at variance.

RESULTS

One hundred twenty breaths of smoke from burning cotton resulted immediately after exposure in a carbon monoxide level of 69.3 ± 4.75 (SE)% in the smoke-exposed group, 66.57 ± 5.12% in the bronchial artery ligation plus smoke group, and 70.7 ± 5.3% in the pulmonary artery ligation plus smoke group. The animals were maintained on an inspired O₂ fraction of 0.5, and carboxyhemoglobin returned to baseline by the end of the experiment.

Gross examination of the lungs at death showed traces of methylene blue in the peripheral lung tissue in all injected animals, thus proving the ligation of the bronchial and not the esophageal artery.

Baseline pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac output, and pulmonary vascular resistance were as follows: 13 ± 1.1 mmHg, 6 ± 0.8 mmHg, 2.8 ± 0.3 l/min, and 2.6 ± 0.7 mmHg·l⁻¹·min in bronchial artery ligation controls; 13 ± 1.1 mmHg, 6 ± 0.4 mmHg, 2.8 ± 0.2 l/min, and 2.8 ± 0.3 mmHg·l⁻¹·min in smoke-inhalation animals; and 14 ± 0.8 mmHg, 6 ± 0.2 mmHg, 3.0 ± 0.1 l/min, and 2.8 ± 0.3 mmHg·l⁻¹·min in smoke-exposed plus bronchial artery ligation animals. No significant change occurred in pulmonary hemodynamics over most of the course of 4 h, although pulmonary artery pressure tended to rise in all groups and cardiac output tended to fall. By 180 min, pulmonary vascular resistance did rise significantly to 4.2 ± 0.5 mmHg·l⁻¹·min in the smoke group and by 210 min to 5.0 ± 0.9 mmHg·l⁻¹ in the bronchial artery ligation plus smoke group. Peak inspiratory pressure as measured proximal to the endotracheal tube was unchanged. In the right pulmonary artery ligation group, the initial main pulmonary artery pressure averaged 9 ± 2 mmHg preligation and rose to 12 ± 2 mmHg immediately postligation. It then slowly drifted back to 8 ± 1 mmHg by 150 min postligation.

Lung lymph flow did not change following bronchial artery ligation in the non-smoke-exposed controls (Fig. 1A). In sheep exposed to smoke, the average lung lymph flow increased dramatically (from 2.4 ± 62 to 9.14 ± 0.9 ml/0.5 h) starting immediately after exposure, with lymph flow increasing to some degree in all animals within the group (Fig. 1B). For the whole group, ligation of the bronchial artery before smoke resulted in a delayed and diminished rise in lymph flow (from 2.8 ± 0.7 to 6.5 ± 10.5 ml/0.5 h), not reaching significance until 3 h after smoke inhalation (Table 1, Fig 1A). The individual data in that group showed little elevation in lymph flow in three of the animals, whereas lymph flow increased similarly to that in the nonligated sheep in the other three animals (Fig. 1C).

Lymph-to-plasma protein ratio (L/P) did not change over time in any group except at 240 min after smoke exposure, when it rose in the smoke and smoke plus bronchial artery ligation groups (Table 1). The same three animals that showed an increase in lymph flow showed an increase in L/P, whereas the other three animals were largely unaffected. Changes in lymph protein flux, calculated as lymph flow × lymph protein concentration, as well as protein flux ratio, calculated as lymph flow × L/P, were significantly increased by smoke inhalation and were delayed and diminished by bronchial artery ligation, consistent with changes in lymph flow (Table 1).

Blood-corrected EVLW/DW was significantly increased following smoke exposure (5.1; P < 0.05) compared with bronchial artery ligation controls (4.1; Fig. 2). At 240 min after smoke inhalation, bronchial artery ligation prevented any increase in lung water.
DISCUSSION

Inhalation of smoke from burning cotton induced a prompt and significant increase in lung lymph flow (Fig. 1A) with no significant rise in pulmonary artery pressure and with no fall in L/P (Table 1), consistent with a microvascular permeability injury as we and others have previously noted (13, 15, 16). Bronchial artery ligation delayed the onset and diminished the magnitude of the increase in lung lymph flow after smoke inhalation (Table 1, Fig. 1A) and significantly reduced the EVLW (Fig. 2). Thus the acute pulmonary edema following inhalation of smoke from burning cotton, which contains many potential edematogenic toxins, one of which is acrolein, can be significantly reduced by bronchial artery ligation, as our laboratory has previously noted with inhalation of synthetic smoke containing only carbon and acrolein (11). Indeed, ligation of the pulmonary artery to the right lung before smoke inhalation, while the bronchial artery was intact, failed to decrease the ensuing smoke-induced pulmonary edema. This does not prove that the pulmonary artery microcirculation did not become a source of edema in these animals, as there is abundant cross circulation between the bronchial arteries and the pulmonary microcirculation, even some precapillary anastomosis (7, 8). Lakshminarayan et al. (17) showed in dogs that the bronchial artery circulation from central airways mainly drained into the systemic circulation, whereas that from the intraparenchymal airways more commonly drained to the pulmonary circulation. Our own data and that of Kimura et al. (16) show that smoke inhalation injures both central and peripheral airways. Thus the potential is there for the car-

### Table 1. Lung lymph parameters before and after bronchial artery ligation and/or smoke inhalation

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Lymph flow, ml</th>
<th>L/P</th>
<th>Flow × L/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.8 ± 0.3</td>
<td>1.0</td>
<td>2.8</td>
</tr>
<tr>
<td>30</td>
<td>2.9 ± 0.4</td>
<td>1.0</td>
<td>2.9</td>
</tr>
<tr>
<td>60</td>
<td>3.1 ± 0.5</td>
<td>1.0</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. L/P, lymph-to-plasma protein ratio; lymph flux is lymph protein flux; flow × L/P is protein flux ratio. *P < 0.05 compared with 30 min. Differences in lymph flow were significant at 120, 150, 180, 210, and 240 min.

Fig. 2. Blood-corrected extravascular lung water-to-dry weight ratio 4 h after 120 breaths of cotton smoke with or without BAL and after 120 breaths of smoke with right pulmonary artery ligation (PAL) or left nonligated lung (NON PAL). Cont. control. *P < 0.05 vs. BAL control.

(4.4; Fig 2). The ratio in the three smoke-exposed and ligated animals that experienced little change in lymph flow after smoke (Fig. 1C) was 3.87 (range 3.51–4.57), whereas the ratio in the sheep in which the lymph flow still rose after smoke and ligation was 5.19 (range 4.7–5.38). The regression of lymph flow vs. EVLW/DW was 0.865 (P < 0.03). In the seven sheep in which the right pulmonary artery was ligated, the main pulmonary artery pressure rose 3 ± 0.3 mmHg, where cardiac output and pulmonary capillary wedge pressure were unchanged. The EVLW/DW was 5.53 ± 0.8 in the ligated lung and 5.01 ± 0.7 (P = 0.09) in the nonligated lung. This compares with 4.1 in three pulmonary artery ligation control lungs (range 3.9–4.3).
However, this may not apply to the right lung. Sakurai et al. (23) have shown that the pulmonary microcirculation permeability is increased after smoke inhalation, at least in part because of an intact bronchial circulation. Nevertheless, there must have been a fall in pulmonary microcirculatory pressure in the right pulmonary artery ligated lung. Hinder et al. (14) showed that pulmonary artery pressure in the left lung after ligation of that artery fell to approximately the pulmonary capillary wedge pressure. That fall in pressure alone should have reduced edema in the right lung of our sheep, if the pulmonary microcirculation were the main site of the smoke injury. However, that lung got as wet as the smoke-exposed nonligated controls. Doubling blood flow and raising pulmonary artery pressure in the nonligated left lung might also have been expected to more markedly increase edema in that lung if the main site of injury were the pulmonary microcirculation rather than the bronchial, and this did not occur. We cannot eliminate the possibility that, as pulmonary artery pressure fell in the ligated lung, bronchial artery flow increased, bringing even more toxic compounds from the smoke-injured lung to increase the permeability of the pulmonary microcirculation more severely. However, Barie and Malik (5) have previously shown that bronchial artery flow does not increase in a normal lung with a ligated pulmonary artery.

Previous studies with cotton smoke inhalation, which demonstrated the prompt increase in bronchial artery flow, did not find a significant rise in lung lymph flow for 8 h, although a trend was present at 4 h. These investigators did show that a bronchial artery ligation produced a delay in the onset of the increase in lymph flow to 12 h after smoke inhalation and reduction in the quantity (1, 2). The reason the previous investigators did not find the early rise in lymph flow after smoke inhalation that we did may relate to their focus on delayed lung injury at 24 h. They awakened the sheep after smoke inhalation to enable it to be a chronic model, and this process may disrupt the early lymph collections. We also used more smoke, although our laboratory has previously shown that 48 breaths of cotton smoke will increase lung lymph flow acutely but not to the same magnitude as 120 breaths (15).

The variation in success in blocking pulmonary edema from smoke inhalation by bronchial artery ligation was striking, with one-half of the sheep showing no response and one-half completely blocked. This may mean that in some animals the pulmonary rather than the bronchial circulation was responsible for the lung edema. However, it is also well documented that the bronchial artery coming off the bronchoesophageal artery supplies, in different sheep, anywhere from 50 to 88% of total systemic arterial supply to the lung and airways (6, 7, 17). Hinder et al. (14) have shown, using a left pulmonary artery pouch septum, that most of the increase in bronchial blood flow to the left lung after smoke inhalation is from the bronchoesophageal artery. However, this may not apply to the right lung. Sakurai et al. (23) have shown that ligation of the bronchoesophageal artery, as we did, is only partially successful at eliminating bronchial artery flow to the lung compared with ethanol sclerosis, which apparently blocks collateral flow into the bronchoesophageal artery. Even when they sclerosed the bronchoesophageal artery as the most effective way to occlude bronchial circulation to the lung, they found a difference in edema accumulation between the right and left lung after smoke inhalation, suggesting, perhaps, that there was a variation in the source of bronchial artery supply to the two lungs (23). Other systemic sources of blood to the lung are the tracheal bronchial artery on the right and the thoracic tracheal branch on the left (6, 19). Thus the animals showing a dramatic response to ligation of the bronchial artery coming off the common bronchoesophageal trunk may have been those for which that vessel was the dominant source of systemic arterial supply to the lung.

We have not eliminated the possibility that the bronchial arteries deliver toxic substances from the smoke-injured airways to the pulmonary microcirculation and that the leak is from the pulmonary microcirculation more than from the bronchial arteries when the pulmonary circulation is perfused. We have, however, shown that the bronchial arteries can account for the edema formation in the absence of much flow or pressure in the pulmonary circulation. Wagner et al. (27) also found that the bronchial circulation could make a major impact on lung lymph flow. By increasing bronchial artery flow threefold, they obtained a 35% increase in lung lymph flow within 90 min. This increase in bronchial artery flow is small compared with the 900% increase in bronchial artery flow that Abdi et al. (1) measured 20 min after inhalation of smoke from burning cotton. The pressure changes in the bronchial artery microcirculation may not be comparable, however, as Wagner et al. (27) increased bronchial artery pressure by over twofold, whereas in smoke inhalation the systemic pressure usually does not increase after smoke inhalation. The increased bronchial artery flow is due to decreased bronchial artery resistance (14). This will likely increase microvascular pressure in the bronchial artery, but this is unknown. Wagner et al. (27) also showed that bradykinin infusion in bronchial arteries to increase vascular permeability produced a doubling of lung lymph flow, which was not seen when bradykinin was infused into the pulmonary circulation. They did not test the combined effect of increasing bronchial artery flow and vascular permeability. We know from tracer studies that acrolein smoke inhalation increases permeability of bronchial circulation (11). Presumably then the combination of increased bronchial artery flow of 900% plus an increase in bronchial circulation permeability could account for a substantial amount of the increase in lung lymph and pulmonary edema after smoke inhalation.

In conclusion, the bronchial circulation may play a major role in the pulmonary and airway edema occurring acutely after inhalation of smoke from burning cotton as 1) ligation of the bronchial arteries substan-
tially reduces the edema and 2) ligation of the pulmonary artery fails to reduce the pulmonary edema.

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