

Airway Obstruction in Sheep with Burn and Smoke Inhalation Injuries

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The goals of this study were (i) to compare the degree and (ii) temporal changes in airway obstruction in sheep with pulmonary injury induced by smoke inhalation and/or burn; (iii) to qualitatively assess the cellular and mucous content of obstructive material; and (iv) to statistically assess a possible relationship between the degree of airway obstruction and pulmonary dysfunction. Using masked histologic slides, we estimated the degree of luminal obstruction in all cross-sectioned airways. The mean degree of bronchial, bronchiolar, and terminal bronchiolar obstruction was significantly greater in animals with smoke injury alone or combined smoke inhalation and burn (S+B) injury, compared with animals with burn injury alone or uninjured animals ($P < 0.05$). In S+B animals, the degree of bronchial obstruction was maximal at 24 h, with a progressive decrease over 72 h. In contrast, the degree of bronchiolar obstruction increased over time. Qualitatively, bronchial casts were largely composed of mucus at early times after injury, whereas neutrophils were the principal component of bronchiolar obstructive material. Localization of specific mucin subtypes in S+B tissues suggests that increasing bronchiolar obstruction is derived, in part, from upper airway material. Multiple linear regression analysis of airway obstruction scores compared with $Pa_{O_2}/F_{I_{O_2}}$ values showed a correlation coefficient of $r = 0.76$, with bronchial and bronchiolar scores predictive of $Pa_{O_2}/F_{I_{O_2}}$ ($P < 0.05$). These results suggest that strategies to remove or decrease formation of upper airway obstructive material may reduce its deposition into small airways and parenchyma and may improve respiratory function in victims of smoke inhalation injury.

The combination of smoke inhalation and burn injuries in sheep models the pathophysiology of these injuries in humans (1). In both sheep and humans, inhalation injury increases the morbidity and mortality rates seen with burn injury alone. Initial injury from smoke inhalation is limited to the trachea and bronchi, and is characterized by mucosal hyperemia and increased microvascular permeability, exfoliation of the epithelial lining, mucous secretion, and an acute inflammatory cell influx. Together, these responses contribute to the formation of obstructive casts, which may cause respiratory distress in patients with severe inhalation injury (2).

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Abbreviations: alcian blue, AB; periodic acid Schiff, PAS; smoke inhalation injury and burn injury, S+B.

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Previous reports have described airway casts following inhalation injury; however, they have been qualitative and limited in number. Initial reports by Herndon and coworkers (1, 3) described casts as limited principally to the trachea and bronchi in an ovine model of cooled cotton smoke inhalation injury. A more recent study by Hubbard and colleagues (4), also using an ovine model, described casts as extending into small bronchioles following inhalation injury. These authors suggested that obstructive airway casts may promote bronchopneumonia and cause alveolar hypoxia (4).

Obstructive casts have been recognized as potentially life threatening in humans (5). In a patient with smoke inhalation injury, acute hypoxemia, sufficient to produce cyanosis, was attributed to an obstructive cast (2). Removal of the cast resolved the critical situation. In another patient with smoke inhalation injury, acute respiratory distress syndrome and high airway pressures developed over several days following injury. Removal of a bronchial cast reduced airway pressures almost immediately, and arterial oxygen tension returned to normal (6). The importance of obstructive casts in treatment of inhalation injury was also discussed by Pruitt and Cioffi (7). These authors suggested that obstructive airway casts following smoke inhalation may promote atelectasis, pneumonia, and barotrauma. These reports underscore the importance of cast formation in the pathophysiology of inhalation injury; however, this potentially important pathologic process has received relatively little attention. Although this study focuses on airway obstruction after inhalation injury, reports have also described airway obstructive casts in humans with other conditions, including asthma, pneumonia, other airway diseases (8), and congenital cardiac malformations (9, 10). This study may contribute to further understanding of airway obstruction in humans without inhalation injury. The aims of this study were: (i) to compare the extent of airway obstruction in animals 48 h after burn injury alone, smoke inhalation injury alone, and combined smoke inhalation and burn (S+B) injury; (ii) to assess the temporal changes in the extent of airway obstruction at 24, 48, and 72 h in sheep with S+B injury; (iii) to describe the cellular and mucous composition of obstructive casts in animals with S+B injury; and (iv) to statistically assess a possible relationship between the degree of airway obstruction and pulmonary function using linear regression analysis.

Materials and Methods

The studies reported here were approved by the Animal Care and Use Committee of the University of Texas Medical Branch, and were conducted in compliance with the guidelines for the care and use

of laboratory animals of the National Institutes of Health and the American Physiology Society. Animals used in this study were female, range-bred adult sheep weighing approximately 40 kg. Preparation for chronic study, and protocols for smoke and burn injury, have been described previously (1, 3, 11). Briefly, the animals were surgically prepared for study under endotracheal halothane anesthesia. The right femoral artery and vein were cannulated, a thermodilution catheter was introduced through the right common jugular vein into the pulmonary artery, and a silastic catheter was positioned in the left atrium to measure left atrial pressure. Following surgical preparation, the animals were allowed to recover for 5–7 d and given free access to food and water. Before injury, the animals were deeply anesthetized with 3% halothane.

Injury Protocols

Smoke inhalation injury was induced in anesthetized sheep by placing 40 g of ignited cotton toweling in a modified bee smoker attached to a tracheostomy tube containing a thermistor. The temperature of the smoke was continuously monitored to ensure that it did not exceed 40°C. Four series of 12 breaths (total 48 breaths) were delivered. Carboxyhemoglobin concentration in the arterial blood was monitored (CO-Oximeter 282; Instrumentation Laboratory, Lexington, MA) after each 12 breaths to ensure that each animal had received an equivalent dose.

Cutaneous burn injury, 3rd degree, was inflicted on anesthetized sheep with a Bunsen burner, with each flank receiving a 20% total body surface area burn. This produces a full-thickness injury with necrosis of the epidermis and dermis, including sensory nerve endings.

To compare the extent of obstruction between different injury protocols, uninjured sheep with surgical preparation ($n = 5$), sheep with burn injury alone ($n = 6$), sheep with smoke inhalation injury alone ($n = 6$), and sheep with S+B injury ($n = 7$) were killed 48 h after injury. To assess the changes in airway obstruction over time, sheep with S+B injury also were killed at 24 ($n = 5$) and 72 ($n = 6$) h after injury.

Resuscitation

Following injury, animals were given free access to food. Water intake was restricted, and the animals were resuscitated with Ringler's lactate solution using the Parkland formula of 4 ml/kg/h/% body surface burned (12). During the study period, the animals were monitored in a critical care facility. Blood gases were monitored to maintain normal pulmonary arterial oxygen tension. Ventilation was performed with a positive end-expiratory pressure of 5 cm H₂O and a tidal volume of 15 ml/kg. During the first 3 h following injury, the inspired O₂ concentration was maintained at 100% and the respiratory rate was kept at 30 breaths/min, to induce rapid clearance of CO after smoke inhalation. After 3 h, ventilation was adjusted according to blood gas analysis to maintain the arterial O₂ saturation above 90% and the PCO₂ between 25 and 30 mm Hg. PaO₂/FI₂ was determined at 3-h intervals until killing.

Tissue Collection and Histologic Analysis

Animals were killed by giving an overdose of ketamine followed by intravenous injection of a saturated potassium chloride solution. After verification of death, the trachea and lungs were removed and tissue for histology was collected in a systematic manner. A 1-cm slice was taken through the middle of the lower lobe of the right lung. The parenchyma was slowly injected with 10% formalin at several sites using a 21-gauge needle and syringe. The slice was then immersed in fixative for 3–5 d. Following fixation, the tissue slice was sampled into a minimum of three blocks by a technician

unaware of the study group. The intrapulmonary portion of the lower lobe bronchus was included in one block, and other tissue samples were taken in a standardized manner between the lobar bronchus and the pleural surface farthest from it. Planimetry of tissues from eight sheep showed that $38.5 \pm 8.58\%$ (mean \pm SD) of the midsection slice area was sampled using this protocol. Paraffin-embedded samples were sectioned at 4 μ m and stained with hematoxylin and eosin. In addition, slides from uninjured and S+B injured animals were stained with alcian blue (AB) at pH 2.5 and the periodic acid Schiff (PAS) stain for the localization of acidic and neutral mucins, respectively (13). To determine the pattern of acidic and neutral mucin distribution in the airway epithelium and obstructive cast material of uninjured sheep and in sheep with S+B injury, masked slides were examined, and the pattern of each mucin subtype was identified by two observers.

Measurement of Airway Obstruction

For quantitation of airway obstruction, all histologic slides were randomly sorted and masked before observation by a single individual. Each slide was systematically scanned using a 4 \times objective, and for each cross-sectioned airway, a score of 0–100% was made as an estimate of the degree of luminal obstruction. Each airway also was classified as a bronchus, a bronchiole, or a terminal bronchiole. Bronchi were defined as airways with supporting cartilage and/or mucous glands. Bronchioles were defined as airways lacking cartilage and mucous glands. Terminal bronchioles were identified as having short, cuboidal lining epithelial cells and limited connective tissue underlying the epithelium. Examples of each type are shown in Figures 1A, 1C, and 4B. The main lobar bronchus was excluded from the analysis, because cast material in this large airway often was displaced during tissue collection and sampling.

After all slides had been examined, mean airway obstruction scores were determined for each animal. Slide codes were then unmasked and scores from each animal were grouped according to their respective injury. Mean obstruction scores were determined for each time post injury by averaging the means from each animal. Nonparametric, Wilcoxon rank sum tests or Student's *t* tests (S-Plus Software; Mathsoft Inc., Seattle, WA) were used to test differences between group means, with statistical significance accepted at $P < 0.05$.

To assess a possible relationship between obstruction scores and pulmonary function, mean bronchial, bronchiolar, and terminal bronchiolar scores for each animal were compared to measurements of the last PaO₂/FI₂ determined before killing. Linear regression models (SigmaStat; SPSS Science, Chicago, IL) were used to determine a correlation coefficient for airway obstruction scores versus PaO₂/FI₂.

Results

In slides from sheep uninjured except for surgical preparation, 43 bronchi were identified, which had an estimated degree of obstruction of $2.7 \pm 2.4\%$ (mean \pm SD). Two hundred seventy bronchioles had an estimated degree of obstruction of $1.6 \pm 0.9\%$, and 52 terminal bronchioles had an estimated degree of obstruction of 0.0%. In all airways of this group, the obstructive material was predominantly noncellular, amorphous, eosinophilic material containing, at most, a few cells.

In animals killed at 48 h after injury, mean degrees of obstruction of bronchi were $4.4 \pm 3.5\%$ in the group with burn injury alone, $18.1 \pm 10.1\%$ in the smoke inhalation injury group, and $29.3 \pm 15.1\%$ in the S+B group. Mean

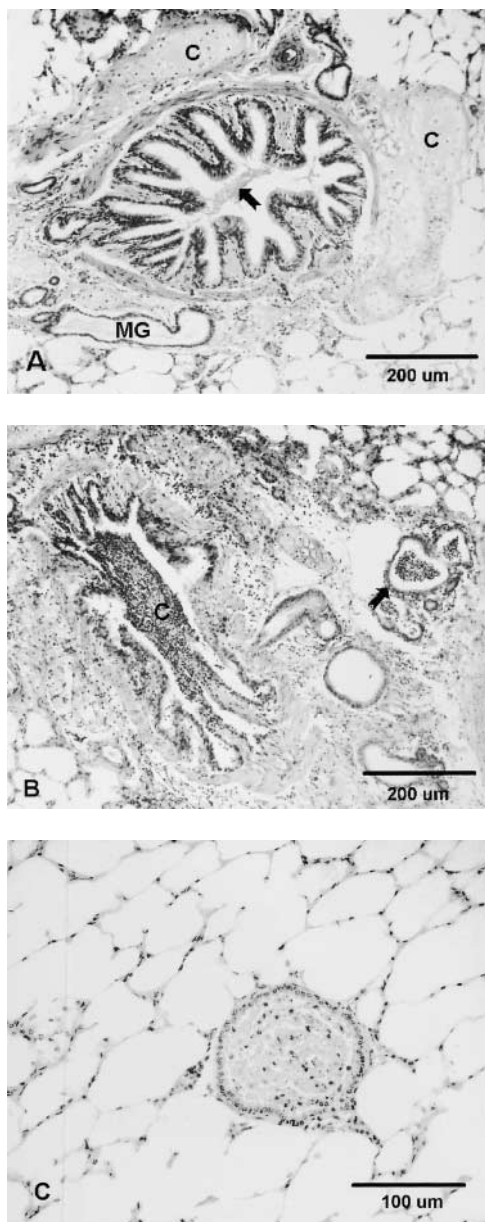


Figure 1. Light micrographs of H&E-stained tissue, showing obstructed airways 48 h after different injury protocols. (A) A bronchus from an animal after burn injury alone. A noncellular, eosinophilic material (arrow) is present within the airway lumen. Cartilage (C) and mucous glands (MG) surround the airway. This airway was scored as 10% obstructed. (B) A bronchus from an animal after smoke injury alone shows approximately 50% obstruction. The obstructive cast material (C) is composed of neutrophils in an eosinophilic matrix. Aggregates of neutrophils (arrow) are evident in mucous glands. (C) A terminal bronchiole scored as 100% obstructed.

scores for bronchi and bronchioles in the smoke alone and S+B groups were significantly greater than the mean scores in burn alone or uninjured animals ($P < 0.05$). Although the mean scores for the S+B group were greater than the smoke alone group, the difference was not statistically sig-

TABLE 1

Mean levels of airway obstruction in uninjured sheep and 48 hours after burn, smoke inhalation, and combined smoke inhalation and burn injury

Injury	Airway Level		
	Bronchi	Bronchioles	Terminal Bronchioles
Uninjured ($n = 5$)	$2.7 \pm 2.4\%$	$1.6 \pm 0.9\%$	$0.0 \pm 0.0\%$
Burn alone ($n = 6$)	$4.4 \pm 3.5\%$	$2.5 \pm 1.5\%$	$0.04 \pm 0.1\%$
Smoke alone ($n = 6$)	$18.1 \pm 10.1\%^{*†}$	$8.1 \pm 3.0\%^{*†}$	$0.3 \pm 0.4\%^{*}$
S+B ($n = 7$)	$29.3 \pm 15.1\%^{*†}$	$11.5 \pm 6.7\%^{*†}$	$1.2 \pm 1.9\%^{*}$

Data presented as mean percent \pm SD (n = number of animals in each group).

^{*}Significantly different from uninjured animal means, Wilcoxon rank sum test, $P < 0.05$.

[†]Significantly different from burn injury, Wilcoxon rank sum test, $P < 0.05$.

nificant. At the terminal bronchiolar level, obstruction scores for the smoke alone and S+B injury models were significantly greater than those for the uninjured group. Table 1 summarizes the data from each injury group. Qualitatively, the obstructive material in the smoke only group included many more inflammatory cells than that in the burn only group. Figure 1A is a light micrograph showing bronchial obstructive material in a sheep with burn injury alone. Figure 1B is a light micrograph of a bronchus from an animal with smoke injury alone, showing the extensive neutrophil content of the obstructive cast. Figure 1C is a light micrograph of a terminal bronchiole scored 100% obstructed, from an animal with S+B injury. Only an occasional terminal bronchiole contained obstructive material; most were scored as 0% obstructed.

In the temporal assessment of airway obstruction in animals with S+B injury, 53 cross-sections of bronchi were identified in tissue 24 h after injury. The mean degree of obstruction was $34.4 \pm 12.9\%$. This was significantly different from uninjured animals ($P = 0.003$). Also in this group, 295 bronchioles and 138 terminal bronchioles were identified with mean obstruction scores of $6.4 \pm 3.9\%$ and $0.1 \pm 0.2\%$, respectively. Mean levels of obstruction of bronchioles and terminal bronchioles were significantly greater than those of the uninjured group ($P < 0.05$). Of the five animal tissues examined at this time period, tissue from four of the animals exhibited at least one bronchial obstruction score of 90–100%, demonstrating a similar pattern of obstruction between animals. In the one remaining animal of this group, the maximum level of bronchial obstruction observed was 20%. Morphologically, most bronchial obstructive material was composed of an eosinophilic, amorphous material, but included varying numbers of neutrophils. Figure 2A shows a light micrograph of a bronchial airway that was scored as 100% obstructed, from tissue 24 h after S+B injury.

In the group studied 72 h after S+B injury, a total of 47 bronchi, 479 bronchioles, and 105 terminal bronchioles were identified. The estimated degree of obstruction was $17.6 \pm 13.0\%$ for bronchi, $12.7 \pm 8.5\%$ for bronchioles, and $0.2 \pm 0.2\%$ for terminal bronchioles. Statistical assessment of the mean bronchial obstruction score compared to the

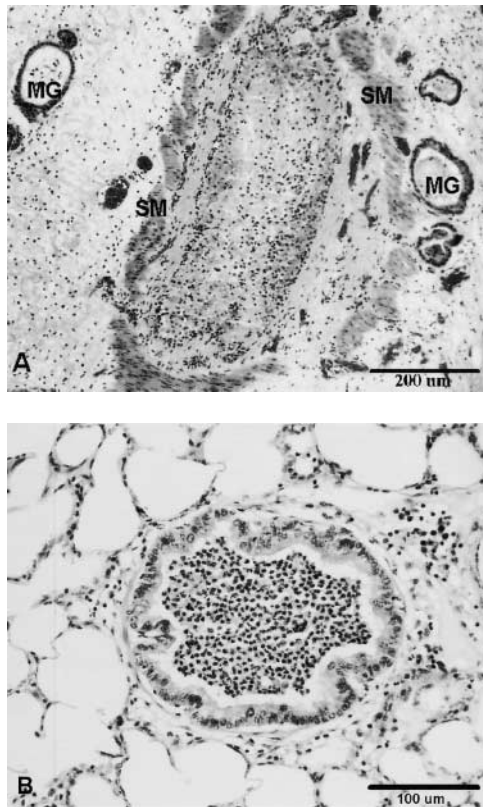


Figure 2. Light micrographs of H&E-stained tissue, showing airways at different times after S+B injury. (A) A bronchial airway, scored as 100% obstructed, from an animal 24 h after injury. Neutrophils and eosinophilic material compose the obstructive material. Mucous glands (MG), with neutrophils in their lumens, and smooth muscle tissue (SM) surround the airway. (B) A bronchiole from an animal 72 h after injury showing obstructive material predominantly composed of neutrophils. This airway was estimated as 80% obstructed.

mean score from the uninjured tissue, with the nonparametric rank sum test, showed a nonsignificant difference ($P = 0.052$). However, following normality and equal variance tests, a significant difference was demonstrated with a t test ($P = 0.033$). Comparison of the degree of obstruction in bronchioles of injured animals with the mean degree of obstruction in uninjured animals with the rank sum test showed a significant difference ($P = 0.004$). Comparison of terminal airway obstruction scores showed no significant difference ($P > 0.05$). Assessment of the variability in the maximum degree of bronchial obstruction between animals showed that from the six animal tissues examined at this time point, tissue from three showed at least one bronchial airway scored as 80–85% obstructed. In tissue from the other three animals, the maximal degree of bronchial obstruction observed ranged from 40–60%. Morphologically, casts appeared to have more neutrophils in comparison with casts from tissue at 24 and 48 h, although a similar amorphous matrix was present in all casts. Figure 2B is a light micrograph of a bronchiole scored as 80% obstructed. The obstructive material is predominantly composed of neutrophils in an eosinophilic matrix.

TABLE 2

Summary of mean obstruction scores at different airway levels and times post-injury in sheep with combined smoke inhalation and burn injury

Time (h)	Airway Level		
	Bronchi	Bronchioles	Terminal Bronchioles
0 ($n = 5$)	$2.7 \pm 2.4\%$	$1.6 \pm 0.9\%$	$0.0 \pm 0.0\%$
24 ($n = 5$)	$34.4 \pm 12.9\%^*$	$6.4 \pm 3.9\%^*$	$0.1 \pm 0.2\%^*$
48 ($n = 7$)	$29.3 \pm 15.1\%^*$	$11.5 \pm 6.7\%^*$	$1.2 \pm 1.9\%^*$
72 ($n = 6$)	$17.6 \pm 13.0\%^*$	$12.7 \pm 8.5\%^*$	$0.2 \pm 0.2\%$

Data presented as mean obstruction level in percent \pm SD (n = number of animals).

*Significantly different from noninjured animals, $P < 0.05$.

Table 2 summarizes the data on changes over time in animals with S+B injury. Assessment of mean airway obstruction levels over time showed a trend of decreasing obstruction of bronchi (Figure 3A), and increasing obstruction of bronchioles (Figure 3B). Figure 3C is a histogram of all bronchial obstruction scores at 24 h after injury, when the mean scores were maximal, demonstrating that approximately 10% of the bronchi scored showed degrees of obstruction between 90–100%. Figure 3D is a histogram of bronchiolar obstruction scores at 72 h after injury, when the scores were maximal for bronchioles, showing that approximately 5% of the bronchioles scored showed degrees of obstruction between 70 and 90%.

In assessment of acidic and neutral mucin localization at different airway levels, all bronchi and large bronchioles exhibited both AB- and PAS-positive staining of epithelial secretory cells (Figure 4A) in both uninjured and S+B injured animals. Bronchial mucous glands also contained mucins staining with both AB and PAS. In smaller airways, the expression of acidic mucins by secretory cells was consistently absent. At the terminal airway level, only PAS-positive, neutral mucin was evident in the airway epithelium of all animals (Figure 4B). In tissues with S+B injury, both AB- and PAS-positive mucins were the major components of most bronchial casts. In tissue sampled 48 and 72 h after injury, we frequently observed acidic mucins in the lumens of terminal bronchioles (Figure 4C). Acidic mucins were also present in adjacent alveolar spaces, together with neutrophils (Figure 4D).

Multiple linear regression analysis of mean airway obstruction scores compared with $Pa_{O_2}/F_{I_{O_2}}$ values for all animals, regardless of injury protocols, showed a correlation coefficient of 0.76. However, only bronchial scores ($P = 0.020$) and bronchiolar scores ($P = 0.003$) were found to be predictive of $Pa_{O_2}/F_{I_{O_2}}$. Terminal bronchiolar obstruction scores were not found to be predictive of $Pa_{O_2}/F_{I_{O_2}}$ ($P = 0.83$). A single independent variable regression model showed a similar pattern of obstruction compared to $Pa_{O_2}/F_{I_{O_2}}$, with correlation coefficients of 0.63 and 0.71 for bronchial and bronchiolar obstruction scores, respectively. Figures 5A and 5B are scatter and regression line plots of bronchial and bronchiolar scores plotted against $Pa_{O_2}/F_{I_{O_2}}$ values. Generally, decreases in the ratio of $Pa_{O_2}/F_{I_{O_2}}$ below 300 were associated with greater than 10% obstruction of bronchi or greater than 5% obstruction of bronchioles.

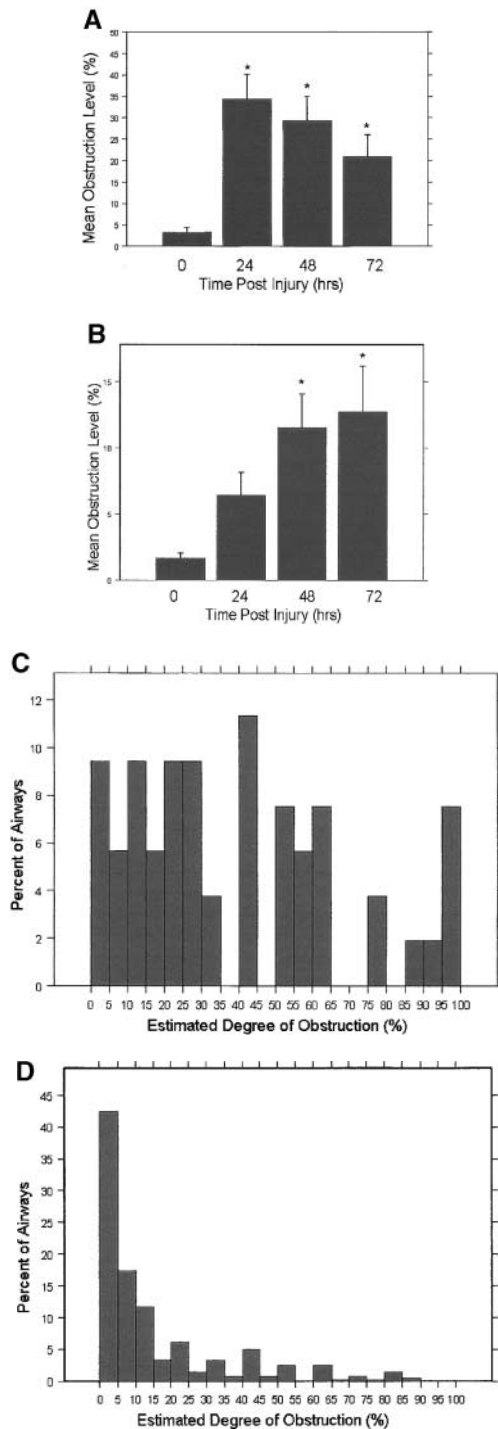


Figure 3. Graphs depicting changes in airway obstruction from animals after S+B injury. (A) Bar graph showing that the mean degree of bronchial obstruction is maximal at 24 h after injury. Bronchial airways show a progressive decrease in mean degree of obstruction over time. (B) Bar graph showing that the mean degree of bronchiolar obstruction increases progressively with time. (C) Histogram of all bronchial obstruction scores 24 h after injury, showing that approximately 10% of these airways were scored as 80–100% obstructed. (D) Histogram of all bronchiolar obstruction scores 72 h after injury, showing that approximately 5% of bronchioles were scored as 70–90% obstructed. *Significantly different from uninjured, $P < 0.05$.

Discussion

This study was done to measure the degree, composition, and location of obstructive material in the airways of sheep after different injury protocols of smoke alone, burn alone, and combined smoke inhalation and burn (S+B) injury. In our first and second study aims, we estimated the degree of luminal obstruction in each cross-sectioned airway identified in histologic sections. This method of measuring airway obstruction was chosen for several reasons. In preliminary measurements of airway obstruction, we used digital imaging techniques to more precisely measure the extent of airway obstruction. However, this method was abandoned because obstructive material often does not have well-defined edges to allow quantitation without considerable error (*uppermost bronchiole* in Figure 4C). In these initial studies, we also found that the time required to collect all variables was prohibitive if many airways were to be studied. In addition to these limitations was the observation that obstructive material is often focal and associated with parenchymal congestion, edema, and inflammation. Because of these limitations, we felt that the best measure of airway obstruction would be achieved by estimating the degree of luminal obstruction of all airways in the tissues collected. Consideration of potential errors in this method suggests that the obstruction scores may underestimate the degree of airway obstruction in sheep after injury. In lung tissue collection, the main bronchial airway in many animals is often extensively obstructed; however, casts from these larger airways are easily displaced in sampling and processing for histologic analysis. Due to possible loss of obstructive material in large airways, we excluded the main bronchus in our study, although other large bronchial airways, which were included in our study, may also have lost obstructive material. Another reason for possible underestimation is the shrinkage of the obstructive material relative to the airway wall. As one can observe in Figures 2B and 4D, the obstructive material has a shape similar to the outline of the airway wall, suggesting that the airway wall, by contact, had shaped the obstructive material. This pattern was observed often in airways with obstruction estimates as small as 50%. This type of artifact may have contributed significantly to a reduction in the percentage of bronchioles that exhibited extensive or complete obstruction. Perhaps the actual configuration of obstructive material and the airway could be better preserved by more elaborate methods of fixation. Another reason that our estimates of airway obstruction may underestimate the degree of obstruction is that obstructive material, examined in longitudinal sections of airways, showed variations in the thickness of the material along the length of the airway. Given this characteristic, randomly obtained airway cross-sections will not show the maximum degree of obstruction.

The first aim of this study was to compare the degree of airway obstruction in uninjured animals to animals with either burn injury alone, smoke inhalation injury alone, or S+B injury. Results showed that 48 h after injury, both the smoke inhalation alone and the S+B injured groups had statistically significant increases in obstruction at each airway level compared with uninjured and burn injured animals. Additionally, across all three airway levels (bronchial,

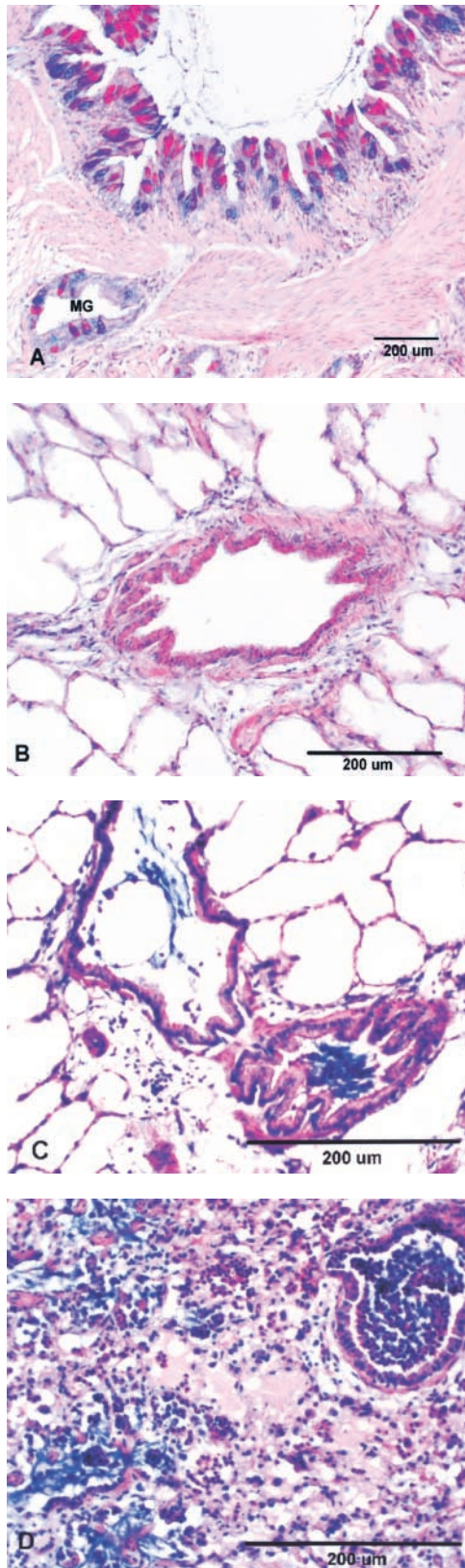


Figure 4. Light micrographs showing alcian blue (AB)- and periodic acid Schiff (PAS)-stained tissue, counterstained with hematoxylin, after S+B injury. (A) A bronchus from an animal without injury. Acidic, AB-stained mucins are blue; neutral, PAS-stained

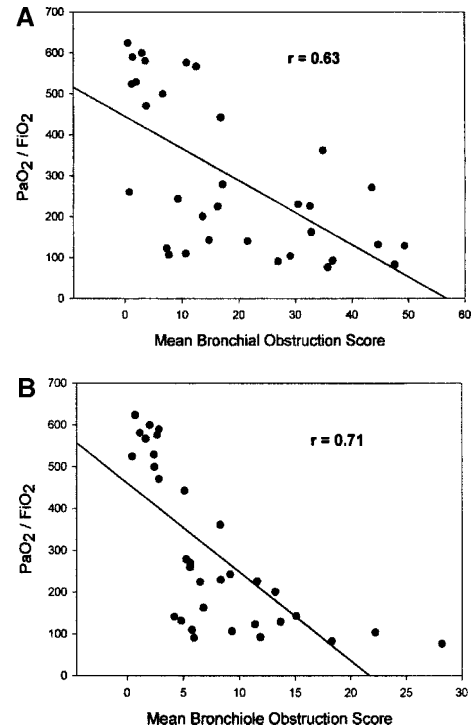


Figure 5. Scatter and regression line plots of obstruction scores from each animal versus its last PaO₂/FiO₂ score before killing. (A) Bronchi. (B) Bronchioles. *r* = Correlation coefficient.

bronchiolar, and terminal bronchiolar), the mean scores from the S+B and burn alone injury models showed a trend of more obstruction relative to smoke injury alone and uninjured animals, respectively (Table 1). Although the extent of obstruction in burn only injured animals was not significantly greater than in uninjured animals, the pattern of increased obstruction suggests that burn injury promotes processes such as increased vascular permeability and mucous secretion that add to the responses that contribute to airway obstruction in smoke inhalation injury.

The second goal of this study was to assess the temporal changes in the degree of airway obstruction in sheep with S+B injury. Results showed that bronchial obstruction was

mucins are red. Both neutral and acidic mucins are evident in the lining epithelium and in the mucous glands (MG). (B) A small bronchiole in tissue from an uninjured animal. Consistently, at these smaller airway levels, only neutral, PAS staining mucin was evident within the lining epithelial cells. (C) Micrograph of tissue 72 h after S+B injury shows two small bronchioles with acidic, AB-stained mucin within their lumens. Lining epithelial cells contain only neutral, PAS-stained mucin. Localization of AB-stained mucins in these airways suggests that obstructive material from the upper airways has migrated distally into the small airways. (D) Micrograph showing acidic mucins (AB), within the lumen of an obstructed bronchiole and within alveolar spaces. Staining of this mucin subtype in the parenchyma co-localized with neutrophils within alveolar spaces.

maximal at 24 h after injury, with a progressive decrease in mean levels of obstruction with time. At this time point, approximately 10% of the bronchi observed were scored as 90–100% obstructed. In contrast, bronchiolar obstruction showed a progressive increase in mean degrees of obstruction, reaching its maximal level 72 h after injury. Approximately 5% of the airways were scored as 70–90% obstructed at 72 h after injury. The mean degree of obstruction in terminal bronchioles showed a statistically significant difference from uninjured tissue at 24 and 48 h. We believe a major reason for the small degree of obstruction and the lack of statistical significance at 72 h is due to the greater cross-sectional area of smaller airways in the lung compared with larger airways. Thus, obstructive material in the larger bronchioles that migrates distally would be distributed in a greater total volume. Another possibility is that our model of cooled cotton smoke exposure did not produce enough small particles for significant deposition into the terminal airways. Particulate matter is an important component for epithelial cell injury in smoke inhalation injury (14–16). Together, these conditions could explain the lack of statistical significance and the pattern of almost total absence of obstructive material in terminal bronchioles, except for an occasional airway, that showed extensive obstruction.

Our third study aim was to assess the expression of acidic and neutral mucins at different airway levels, and to examine these mucins in the obstructive material. In both injured and uninjured sheep, acidic and neutral mucins were present in bronchial and large bronchiolar epithelial secretory cells, as well as in bronchial glands. As the airways decreased in size, epithelial expression of acidic mucins decreased, and only neutral mucins were observed in the epithelium of terminal bronchioles. At 24 h after injury, bronchial obstructive material was largely composed of secreted mucus. A large portion of the mucins appeared to be derived from the bronchial glands, which exhibited almost total depletion of mucus. At later time points, although mucus was still evident, inflammatory cells became the predominant component of the obstructive material. We observed acidic mucins focally within terminal bronchioles and in their adjacent parenchyma after S+B injury. These observations were most common in tissue collected 48 and 72 h after injury. Because acidic mucins are secreted only in bronchi and large bronchioles, its presence in terminal bronchioles after injury suggests that the observed increase in bronchiolar obstruction over time is derived, in part, from migration of upper airway obstructive material into the lower airways. This conclusion is consistent with our earlier observation that smoke inhalation injury causes extensive loss of columnar epithelium in the trachea and large bronchi, which would contribute to defective mucociliary clearance (17). These results suggest that early, aggressive intervention to reduce or remove obstructive material in upper airways may decrease lower airway obstruction and the deposition of mucus, cell debris, and neutrophils into the parenchyma. Additionally, the distal migration of upper airway material into the parenchyma may also promote deposition of trapped bacteria into the parenchyma. The importance of pneumonia is underscored by the study of Shirani and colleagues, who reported that the incidence of

pneumonia in burn patients with inhalation injury was 38%, in contrast to only 9.1% for patients without inhalation injury. These authors also reported that up to 40% of burn patient mortality is related to pneumonia (18).

Although this study demonstrated distal migration of upper airway material, an additional mechanism of increasing small airway obstruction is suggested in unpublished studies from our laboratory showing increased numbers of neutrophils in the epithelium of small bronchi and bronchioles after S+B injury. These preliminary results suggest that neutrophils are migrating across the epithelium, which would also contribute to the increased degree of bronchiolar obstruction.

The fourth aim of this study was to use linear regression methods to assess whether there is a relationship between obstruction scores and pulmonary function. With a multiple regression model, a correlation coefficient of 0.76 was calculated, with both bronchial ($P = 0.02$) and bronchiolar scores ($P = 0.003$) being predictive of Pa_{O_2}/FI_{O_2} . Terminal bronchiolar scores were not found to be predictive of pulmonary function. We suspect that the lack of correlation between terminal airway scores and loss of pulmonary function is due to the limited number of these airways scored per animal and the wide range of their values.

Linear regression analysis with bronchi and bronchioles as single independent variables supported the multiple regression analysis. The rather high correlation coefficients found from these analyses offer support to the concept that airway obstruction contributes to the decrease in pulmonary function after smoke inhalation injury. This conclusion is strengthened by our observation that airway obstruction was localized to areas of more extensive parenchymal injury. Also in a previous study, heparin infusion after smoke inhalation injury reduced cast formation and improved pulmonary function (19).

Several mechanisms exist by which airway obstruction may contribute to the pathophysiology of inhalation injury. The initial morphologic changes after smoke inhalation injury are focal (1, 3, 20), although we suspect that the toxic components in smoke are distributed to the lung in a more uniform manner. Thus, some mechanism must lead to the observed focal distribution of injury. This study has demonstrated extensive variability in the degree of airway obstruction, which may be a major factor in promoting the focal histopathologic changes observed after smoke inhalation injury. Near-total obstruction of a few bronchi would prevent ventilation of individual lung segments (21), whereas partial obstruction would be expected to reduce ventilatory flow. These changes in ventilation could produce hypoxia, which is known to modulate acute inflammatory cytokine production (22–24).

An additional mechanism may lead to focal lung injury. Total obstruction of some bronchi would promote hyperinflation-induced barotrauma of lung segments supplied by unobstructed airways. Hyperventilation has been shown to induce acute inflammatory cytokine production (25, 26). Narimanbekov and coworkers also demonstrated that hyperinflation increased cytokine-dependent lung injury in hyperventilated rabbits (25). However, the degree of hyperinflation-induced barotrauma may have varied somewhat

among the animals used in our study, because the protocol allowed the ventilator rate to be adjusted within fixed limits based on blood gas analysis.

In conclusion, S+B and smoke only injuries produce higher levels of airway obstruction compared with airways of uninjured and burn only-injured animals. In the S+B model, large airway obstruction was greatest 24 h after injury. Bronchiolar obstruction increased during the study period of 72 h. Localization of acidic mucins in the terminal airways and parenchyma demonstrated distal migration of upper airway material. This study demonstrated extensive focal airway obstruction following S+B injury. Such focal obstruction may help to account for the observed multifocal distribution of lung injury. Airway obstruction may be an important factor in causing decreased respiratory function after burn and smoke inhalation injury.

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