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Integrity of Airway Epithelium in Pediatric Burn Autopsies: Association with Age and Extent of Burn Injury

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Abstract

This study examines the structural integrity of the airway epithelium in autopsy tissues from pediatric burn subjects.

Methods—A semi-quantitative score for the degree of airway epithelial integrity was made for seventy- two pediatric burn autopsies. Multivariate ordinal logistic regression was performed to identify relationships between epithelial integrity and conditions related to tissue fixation, time of death after injury, age, total body surface area burn (TBSA), extent of 3rd degree burn injury, presence of inhalation injury, ventilator days and pneumonia.

Results—No significant difference in epithelial integrity scores was identified between burn only cases and those with inhalation injury. Significant correlations with bronchiolar epithelial integrity scores were identified for age, $p = 0.02$, and percent 3rd degree burn injury, $p = 0.02$. There was no significant relationship between epithelial integrity and time between death and autopsy, $p > 0.44$.

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Conclusions—Airway epithelial loss seen in autopsy tissue is not simply an artifact of tissue fixation. The degree of compromise correlates most strongly with age and degree of burn injury. Further studies are needed to identify physiological or critical care factors following burn injury that contribute to compromise in the structural and functional properties of the airway epithelium.

Keywords

trauma; innate defense; pneumonia; mucociliary function; infection

Introduction

Although pneumonia is a major cause of morbidity and mortality of burn subjects, there is limited understanding of how burn trauma and factors in critical care may alter the airway epithelium and its properties of innate defense. Intuitively, necrosis or detachment of airway epithelium would interfere with mucociliary clearance of bacteria, and this important barrier against infection would be compromised. Inhalation of toxic smoke produced inside a burning building certainly has an injurious effect on the airway epithelium, but the possible contributions of other factors in epithelial loss or damage are not clear. In a past study of sixty-two autopsy cases, we identified extensive distal migration of upper airway mucus into the small airways and parenchyma in scald only victims, suggesting dysregulation in the process of mucociliary clearance [1]. In a more recent studies of autopsy tissue, we found that regardless of the presence of inhalation injury, small airways are extensively obstructed and that 36% of the cases showed bacterial invasion of the airway mucosa [2]. Together, these studies suggest compromise in airway epithelial function following burn injury. However, our ability to study airway damage in burn subjects using autopsy tissue is limited, as postmortem detachment of the airway epithelium from the basement membrane is common and this factor must be considered in microscopic analysis.

The purpose of this study was to assess the structural integrity of the airway epithelium in autopsy tissue in relation to several measurable factors that included the time between death and autopsy (TBDA), also known as the postmortem interval in which autolysis may occur, the percentage of total body surface area (TBSA) occupied by 3rd degree burns, the presence of inhalation injury, the time between injury and death, days after injury, number of days with ventilatory support (VD) and presence of pneumonia.

Materials and Methods

Approval for the analysis of autopsy material from these patients was obtained from the University of Texas Medical Branch Institutional Review Board. Lung tissue samples from 72 autopsies performed between 2000 and 2012 were included in the study. From three to seven histological sections of lung tissue were examined from each case. All tissue sections were examined by one person experienced in the study of airway epithelial structure (RAC) without knowledge of any information related to the autopsy case. In cases where there was total absence of an epithelium, the airway was identified by the pattern of basal cells in addition to the presence of surrounding smooth muscle and vasculature, appropriate for the luminal size of the airway. Following scoring for epithelial loss, a pathologist (HKH),

reviewed the slides and confirmed the pattern of airway preservation. For each case, scores were assigned for bronchial and bronchiolar epithelial integrity. A score of 0 indicated that airway epithelium was absent in all sections examined, 1 = an epithelium was present but rarely seen, 2 = the presence of an epithelium either attached or detached but remaining within the airway cross section was commonly seen, and 3 = an epithelium, either still attached to the basement membrane or within the airway lumen, was consistently seen for all airway cross-sections. Following scoring, information related to the patient's age, gender, and race, along with conditions related to the injury and death, TBSA burned, percent third degree burn, presence of an inhalation injury, days after injury at the time of death (DAI), number of ventilatory days (VD) and time between death and autopsy (TBDA) were tabulated with their related airway epithelial scores. The presence of inhalation injury was recorded when this diagnosis was included in the patient's chart prior to death, and was based on conventional clinical criteria including bronchoscopic findings of soot, hyperemia and epithelial sloughing in the upper airways [3,4]. Additionally, for each case, the presence of pneumonia at death was extracted from the autopsy record. Of the 72 cases reviewed, bronchial airways, identified as airways with cartilage or mucosal glands, could not be identified in sections from ten cases.

For statistical analysis, nonparametric, Mann-Whitney rank sum tests were used to assess differences in integrity scores and case demographics between burn only and subjects with an inhalation injury using SigmaPlot software (Systat, San Jose, CA). Multivariate ordinal logistic regression, based upon the proportional odds model with equidistant intervals, was used to model the associations of bronchial and bronchiolar scores with age, total body surface area burn, percentage of 3rd degree burn, presence of inhalation injury, presence of pneumonia, days between injury and death, number of ventilatory days and hours between death and autopsy. Age, days after injury, and hours from death to autopsy were log (base 2) transformed for better centering and to simplify interpretation. The Pearson correlation between age and third degree burn percentage was assessed as an indication of their independence. Multivariate analyses were performed using R statistical software [5] with ordinal logistic regression performed using the “ordinal” package [6]. All statistical tests assumed a 95% level of confidence.

Results

Case Demographics

Figures 1A and 1B are micrographs illustrating examples of the absence and presence of an epithelium in a bronchus and bronchiole respectively. Following scoring of airway integrity, the specifics of each autopsy case were identified. Case demographics and issues related to the burn and inhalation injury, ventilatory days, postmortem interval and pneumonia are shown in Table 1.

Comparison of cases with inhalation injury to those with burn injury alone

Table 2 shows the comparison in the subjects with burn injury alone and those with both burn and inhalation injury. Mean values and standard deviations (SD) associated with TBSA, percent 3rd degree burn, time of death after injury and airway integrity scores for

each group are included. There was a significantly larger TBSA burn ($p = 0.02$) and percent 3rd degree ($p = 0.02$) in subjects with inhalation injury compared to victims with burn injury alone. No significant difference was detected between the two groups in the time between injury and death (days after injury, DAI) ($p = 0.9$), time between death and autopsy (TBDA) ($p = 0.2$) or number of ventilatory days (VD) ($p = 0.3$). Assessment of airway integrity scores showed no significant difference in the scores for degree of bronchial ($p = 0.61$) or bronchiolar airway integrity ($p = 0.52$) between the two groups.

Relationship between histological findings and multivariate injury characteristics

Given that there was no statistical difference in airway integrity scores for victims with inhalation injury compared to those with burn injury alone, multivariate regression was performed for all cases. As summarized in Table 3A, multivariate ordinal logistic regression found no significant relationship between bronchial scores and any of the variables tested ($p > 0.14$). Significant associations were seen between bronchiolar scores and age ($p = 0.02$) and percent third degree burn ($p = 0.02$), as summarized in Table 3B. No other variable showed evidence of a significant relationship with the bronchiolar epithelial integrity scores ($p > 0.17$). Bronchiolar scores tended to decrease with increasing age and increasing third degree burn area. Each doubling of age was associated with a 54% ($1/.65 = 1.54$) increase in the odds of a decreased bronchiolar score. Each 1% increase in third degree burn area was associated with a 2% ($1/.98 = 1.02$) increase in the odds of a decreased bronchiolar score. Graphical presentation of the relationship between age and 3rd degree with bronchiolar integrity scores are shown in figures 2A and 2B, respectively. Due to the established relationship of inhalation injury to airway epithelial exfoliation, open dots are used to show cases with concomitant burn and inhalation injury.

Given that age and extent of 3rd degree burn injury were associated with bronchiolar integrity scores, Pearson correlation was performed with these two variables. A significant relationship between these two variables was identified ($p=.03$), however, the coefficient of determination was low, ($R^2 = 0.07$).

Discussion

This study was conducted using lung tissue from 72 autopsy cases from 2000 to 2012. Histologic study of 43 of these cases was included in a recent publication on autopsies of burn subjects with a focus on the semiquantitative assessment of changes associated with diffuse alveolar damage, the clinical correlate of ARDS [7]. Because changes in the airway epithelium have not been described in ARDS, that study did not include any assessment of the airways. In a previous study of 14 autopsies that were systematically sampled to allow scoring of airway obstruction, the integrity of the airway epithelium was not assessed [2]. The focus in the current study was the airway epithelium alone, as loss of airway epithelium had not been previously examined. The results of the present study show that the absence of an epithelium seen in the histological sections of lung tissue from burn subjects correlates with age and the size of third degree burns, expressed as percent of total body surface area, but lacks evidence of any significant relationship with tissue fixation as measured by the time between death and autopsy. This suggests that the variable degree of epithelial presence

in tissue is not strongly related to autolysis and postmortem detachment, but rather to compromise of epithelial structure related to other factors. Given the importance of the airway epithelium to respiratory health, mechanistic understanding of processes associated with epithelial structural compromise following burn injury could lead to improvements in the critical care of burn victims.

Numerous studies have focused on parenchymal pathology in burn victims using autopsy tissue describing atelectasis, airway obstruction, bronchopneumonia, necrotizing pneumonia, hemorrhage and diffuse alveolar damage, however, limited studies have specifically focused to the airway epithelium. A study by Toor et al. found a relationship of airway lesions with length of survival and a sequence of damage that includes mucosal necrosis and denudation, acute inflammation and ulceration, and squamous metaplasia [8]. In a review of over one hundred autopsy cases, Phillips and Cope concluded that respiratory tract damage was the “principal killer of burn patients”, and that this pathology is often overlooked due to the absence of a systematic examination of the entire respiratory tract during autopsy [9]. A major limitation of these two studies is that no comparison of airway lesions was made between burn victims with and without an inhalation injury.

The results of this study found no significant difference in scores between burn victims with concomitant inhalation injury compared to victims with burn injury alone. Thus, regardless of an inhalation injury, patients who die from burn injury alone show evidence of airway pathology. It is well established in humans with inhalation injury that toxins in smoke cause exfoliation of the upper airway epithelium [10]. This has been confirmed in both rabbit [11] and ovine models of inhalation injury [12,13]. Thus, the absence of a significant change in airway integrity scores detected between burn victims with inhalation injury to those with burn injury alone was surprising and may relate to the time interval between injury and death. In both humans with inhalation injury and in animal models, loss of the epithelium is evident within hours after injury. In the ovine model of airway repair following inhalation injury, within 4 days after denuding of the tracheal epithelium, the airway surface is repopulated with cells, and within two to three weeks a functional epithelium is reestablished [14]. Thus, in this study, where the mean time of tissue assessment was 31 days after injury in victims with inhalation injury (Table 2), the ability to detect greater epithelial compromise in patients with smoke inhalation injury may be limited.

Multivariate analysis identified a relationship of age with airway integrity scores. It is well established that teens, the oldest population in our study, have a lower morbidity and mortality in relation to their degree of burn injury compared to the very young and old [15,16]. Thus, the finding of increased epithelial loss with increasing age was a surprise. As presented, there was a positive correlation between age and greater TBSA burn, although the coefficient of determination was low ($R^2 = 0.07$). Possibly, the relationship between age and TBSA may reflect the admissions policies at our institution. Due to the high risk of mortality in the very young, even victims with low TBSA are admitted, whereas in older adolescents, typically only cases with larger TBSA are admitted. As seen in the regression plot of figure 2A, all cases with an integrity score of zero were from subjects either 14 ($n = 3$), 15 ($n = 1$) or 16 ($n = 2$) years of age. A regression analysis of data from cases less than 10 years of age ($n = 47$) found no correlation between age and TBSA burn ($p = 0.4$) or between age and

bronchiolar integrity score ($p = 0.6$). Based on this post hoc analysis, we suspect that the observed relationship of age and epithelial loss may be influenced by the demographics specific to our institution.

The significant relationship of bronchiolar airway integrity scores with percentage of 3rd degree injury suggests that conditions related to the severity of burn injury lead to compromise in epithelial structural integrity. The absence of a corresponding significant relationship with bronchial scores may be due to sampling limitations. Typically, between one and five bronchi were scored per case, whereas 10 to 100 bronchioles were examined. Thus, the ability to detect a relationship of epithelial loss for different airway levels with other fixed variables would be stronger for bronchioles than for bronchi. As presented in the regression plot of TBSA vs. bronchiolar scores (Fig 2B), the coefficient of determination was $R^2 = 0.15$, thus, there was considerable variability in TBSA relative to the linear model. Although the factors responsible for this variation are not known, they may include airway bacterial load, oxidative stress from the physiological response after burn, supportive oxygen therapy, airway management, use of antibiotics and other drug treatments, hydration status, sepsis and inhalation injury.

The observed changes in epithelial integrity with the extent of burn injury may be related to the severe physiological changes that occur after injury. These changes are well characterized and include hyperglycemia and an increase in circulating pro-inflammatory cytokines, catecholamines and cortisol, a decrease in systemic anti-oxidant potential and a negative nitrogen balance [17-22]. These physiological changes are associated with dysfunction of multiple organs including the lungs [23]. However, the physiologic changes following burn injury that alter pulmonary tissues are difficult to identify. Our current understanding of lung dysfunction from burn injury alone is mostly derived from animal studies. In these reports, systemic inflammation and production of inflammatory cytokines [24-26], neuropeptides [27] and reactive oxygen and nitrogen species [28-30] are associated with pulmonary homeostasis. Typically, burn induced changes in lung function and vascular permeability have been identified by physiologic methods or by examining the extent of lung water and/or Evans Blue dye content, while additional measurements have been focused on inflammatory cell products and cytokines in broncho-alveolar lavage (BAL) or in lung tissue. Recent support that physiological changes following burn injury affect the airway epithelium is shown by Jacob et al. [31]. Using a rat model of scald injury, the authors demonstrate that 24 hours after a scald injury, significant changes in tracheal epithelial cell gene expression were identified, in addition, to a decrease in mucociliary clearance and cell proliferation. An increase in malondialdehyde levels was also detected in the tracheal epithelial cell suggesting the changes were related to oxidative stress [31].

Loss of airway epithelium after burn trauma would be expected to have a significant impact on the health of burn patients. Besides conduction of air, a major function of the airway epithelium is to maintain a sterile lung environment. The functions of the airways in innate defense are accomplished through complex and tightly regulated processes. The airway epithelium is covered with an airway surface layer composed of mucus glycoproteins, antibacterial peptides and a tightly regulated water and electrolyte composition that provide rheological properties essential for cilia to move mucus with captured particulate matter and

pathogens out of the airways [32]. Multiple factors that include both adrenergic and muscarinic receptors [33], in addition, to cytokines and other inflammatory mediators [34-36], nitric oxide [37], endothelin-1 [38,39] and purinergic receptors [40-42] contribute to airway homeostasis and mucociliary clearance. Thus, the physiological perturbations seen with burn injury may modify airway surface layer dynamics. Loss of airway epithelium following burn injury would completely eliminate this important innate defense system.

In the current study, we found no significant evidence of a relationship between airway integrity scores and the incidence of pneumonia ($p = 0.19$ and 0.17 for bronchi and bronchioles, respectively), but a stepwise decrease was present with increasing airway integrity. The percent of cases with pneumonia was 83, 63, 52 and 33 for groups with integrity scores of 0, 1, 2, 3, respectively. Possibly, an increase in the number of autopsy cases reviewed could have established a statistical relationship between epithelial integrity and lung infection.

In conclusion, this study identifies a compromise in the structural integrity of the airway epithelium in burn victims that has not been previously described. The loss of structural integrity correlated strongly with the extent of 3rd degree burn injury and with patient age; suggesting that age, extent of burn injury, and/or factors in their critical care alter airway epithelial structural integrity. A significant limitation of the current study is that only patients who died were examined, so the incidence and degree of airway epithelial damage in burn patients who survive could not be determined. However, development of methods to assess airway epithelial function and integrity in burn victims during critical care may prove beneficial for identification of those with an increased risk of airway and lung related complications. The relationships of trauma induced physiological stress with airway epithelial structure and function is an important area of future investigations.

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References

1. Cox RA, Mlcak RP, Chinkes DL, Jacob S, Enkhbaatar P, Jaso J, Parish LP, Traber DL, Jeschke M, Herndon DN, Hawkins HK. Upper airway mucus deposition in lung tissue of burn trauma victims. *Shock*. 2008; 29:356–361. [PubMed: 17693942]
2. Cox RA, Jacob S, Zhu Y, Mlcak R, Kraft R, Herndon DN, Hawkins HK. Airway obstruction and bacterial invasion in autopsy tissue of pediatric burn victims. *J Burn Care Res*. 2014; 35:148–153. [PubMed: 24503967]
3. Pruitt BA Jr, Cioffi WG. Diagnosis and treatment of smoke inhalation. *J Intensive Care Med*. 1995; 10:117–127. [PubMed: 10155177]
4. Desai MH, Rutan RL, Herndon DN. Managing smoke inhalation injuries. *Postgrad Med*. 1989; 86:69–6. [PubMed: 2587465]
5. R Core Team. R: A language and environment for statistical computing. 2013
6. Christensen RHB. Ordinal-Regression modeling for the ordinal data R package version. 2012; 2012:09–11.

7. Sousse LE, Herndon DN, Andersen CR, Zovath A, Finnerty CC, Mlcak RP, Cox RA, Traber DL, Hawkins HK. Pulmonary histopathologic abnormalities and predictor variables in autopsies of burned pediatric patients. *Burns*. 2015; 41:519–27. [PubMed: 25445004]
8. Toor AH, Tomashefski JF Jr, Kleinerman J. Respiratory tract pathology in patients with severe burns. *Hum Pathol*. 1990; 21:1212–1220. [PubMed: 2249834]
9. Phillips AW, Cope O. Burn therapy. II. The revelation of respiratory tract damage as a principal killer of the burned patient. *Ann Surg*. 1962; 155:1–19. [PubMed: 14486219]
10. Ligen L, Hongming Y, Feng L, Chuanan S, Daifeng H, Xiaoye T. Morphologic changes and prognosis of the respiratory tract epithelium in inhalation injury and their relationship with clinical manifestations. *Surgery*. 2012; 151:206–212. [PubMed: 21899868]
11. Goldman R, Moshonov S, Zor U. Calcium-dependent PAF-stimulated generation of reactive oxygen species in a human keratinocyte cell line. *Biochim Biophys Acta*. 1999; 1438:349–358. [PubMed: 10366777]
12. Abdi S, Evans MJ, Cox RA, Lubbesmeyer H, Herndon DN, Traber DL. Inhalation injury to tracheal epithelium in an ovine model of cotton smoke exposure. *Am Rev Respir Dis*. 1990; 142:1436–1439. [PubMed: 2252264]
13. Jacob S, Kraft R, Zhu Y, Jacob RK, Herndon DN, Traber DL, Hawkins HK, Cox RA. Acute secretory cell toxicity and epithelial exfoliation after smoke inhalation injury in sheep: an electron and light microscopic study. *Toxicol Mech Methods*. 2010; 20:504–509. [PubMed: 20843269]
14. Barrow RE, Wang CZ, Cox RA, Evans MJ. Cellular sequence of tracheal repair in sheep after smoke inhalation injury. *Lung*. 1992; 170:331–338. [PubMed: 1434783]
15. 2012 National Burn Repository: Report of data from 2002–2011. 2014
16. Barrow RE, Spies M, Barrow LN, Herndon DN. Influence of demographics and inhalation injury on burn mortality in children. *Burns*. 2004; 30:72–77. [PubMed: 14693089]
17. Jeschke MG, Mlcak RP, Finnerty CC, Norbury WB, Gauglitz GG, Kulp GA, Herndon DN. Burn size determines the inflammatory and hypermetabolic response. *Crit Care*. 2007; 11:R90. [PubMed: 17716366]
18. Jeschke MG, Chinkes DL, Finnerty CC, Kulp G, Suman OE, Norbury WB, Branski LK, Gauglitz GG, Mlcak RP, Herndon DN. Pathophysiologic response to severe burn injury. *Ann Surg*. 2008; 248:387–401. [PubMed: 18791359]
19. Jutkiewicz-Sypniewska J, Zembron-Lacny A, Puchala J, Szyszka K, Gajewski P. Oxidative stress in burnt children. *Adv Med Sci*. 2006; 51:316–320. [PubMed: 17357333]
20. Vindenes HA, Ulvestad E, Bjerknes R. Concentrations of cytokines in plasma of patients with large burns: their relation to time after injury, burn size, inflammatory variables, infection, and outcome. *Eur J Surg*. 1998; 164:647–656. [PubMed: 9728783]
21. Woolliscroft JO, Prasad JK, Thomson P, Till GO, Fox IH. Metabolic alterations in burn patients: detection of adenosine triphosphate degradation products and lipid peroxides. *Burns*. 1990; 16:92–96. [PubMed: 2350417]
22. McMillen MA, Huribal M, Cunningham ME, Bala RJ, Pleban WE, D'Aiuto ML. Endothelin-1, interleukin-6, and interleukin-8 levels increase in patients with burns. *J Burn Care Rehabil*. 1996; 17:384–389. [PubMed: 8889860]
23. Kraft R, Herndon DN, Finnerty CC, Shahrokhi S, Jeschke MG. Occurrence of Multiorgan Dysfunction in Pediatric Burn Patients: Incidence and Clinical Outcome. *Ann Surg*. 2014; 259:381–387. [PubMed: 23511841]
24. Drost AC, Larsen B, Aulick LH. The effects of thermal injury on serum interleukin 1 activity in rats. *Lymphokine Cytokine Res*. 1993; 12:181–185. [PubMed: 8347765]
25. Peter FW, Schuschke DA, Barker JH, Fleishcher-Peter B, Pierangeli S, Vogt PM, Steinau HU. The effect of severe burn injury on proinflammatory cytokines and leukocyte behavior: its modulation with granulocyte colony-stimulating factor. *Burns*. 1999; 25:477–486. [PubMed: 10498354]
26. Piccolo MT, Wang Y, Sannomiya P, Piccolo NS, Piccolo MS, Hugli TE, Ward PA, Till GO. Chemotactic mediator requirements in lung injury following skin burns in rats. *Exp Mol Pathol*. 1999; 66:220–226. [PubMed: 10486240]

27. Sio SW, Puthia MK, Lu J, Mochhala S, Bhatia M. The neuropeptide substance P is a critical mediator of burn-induced acute lung injury. *J Immunol.* 2008; 180:8333–8341. [PubMed: 18523300]
28. Cevik O, Oba R, Macit C, Cetinel S, Kaya OT, Sener E, Sener G. Lycopene inhibits caspase-3 activity and reduces oxidative organ damage in a rat model of thermal injury. *Burns.* 2012; 38:861–871. [PubMed: 22356815]
29. Fujii T, Otsuka T, Tanaka S, Kanazawa H, Hirata K, Kohno M, Kurihara N, Yoshikawa J. Plasma endothelin-1 level in chronic obstructive pulmonary disease: Relationship with natriuretic peptide. *Respiration.* 1999; 66:212–219. [PubMed: 10364736]
30. Chen LW, Wang JS, Chen HL, Chen JS, Hsu CM. Peroxynitrite is an important mediator in thermal injury-induced lung damage. *Crit Care Med.* 2003; 31:2170–2177. [PubMed: 12973176]
31. Jacob S, Zyu Y, Kraft RCC, Carmical JR, Wood TG, Enkhbaatar P, Herndon DN, Hawkins HK, Cox RA. Physiological and Molecular Changing in the Tracheal Epithelium of Rats following Burn Injury. *Internat J Burns Trauma.* 2015
32. Randell SH, Boucher RC. Effective mucus clearance is essential for respiratory health. *Am J Respir Cell Mol Biol.* 2006; 35:20–28. [PubMed: 16528010]
33. Nadel JA, Davis B. Parasympathetic and sympathetic regulation of secretion from submucosal glands in airways. *Fed Proc.* 1980; 39:3075–3079. [PubMed: 7428952]
34. Borchers MT, Carty MP, Leikauf GD. Regulation of human airway mucins by acrolein and inflammatory mediators. *Am J Physiol.* 1999; 276:L549–L555. [PubMed: 10198352]
35. Cohn L, Whittaker L, Niu N, Homer RJ. Cytokine regulation of mucus production in a model of allergic asthma. *Novartis Found Symp.* 2002; 248:201–213. [PubMed: 12568496]
36. Dwyer TM, Farley JM. Human neutrophil elastase releases two pools of mucinlike glycoconjugate from tracheal submucosal gland cells. *Am J Physiol Lung Cell Mol Physiol.* 2000; 278:L675–L682. [PubMed: 10749744]
37. Nagaki M, Shimura MN, Irokawa T, Sasaki T, Shirato K. Nitric oxide regulation of glycoconjugate secretion from feline and human airways in vitro. *Respir Physiol.* 1995; 102:89–95. [PubMed: 8610212]
38. Blouquit S, Sari A, Lombet A, D'Herbomez M, Naline E, Matran R, Chinot T. Effects of endothelin-1 on epithelial ion transport in human airways. *Am J Respir Cell Mol Biol.* 2003; 29:245–251. [PubMed: 12626340]
39. Sabater JR, Otero R, Abraham WM, Wanner A, O'Riordan TG. Endothelin-1 depresses tracheal mucus velocity in ovine airways via ET- A receptors. *Am J Respir Crit Care Med.* 1996; 154:341–345. [PubMed: 8756804]
40. Button B, Picher M, Boucher RC. Differential effects of cyclic and constant stress on ATP release and mucociliary transport by human airway epithelia. *J Physiol.* 2007; 580:577–592. [PubMed: 17317749]
41. Com G, Clancy JP. Adenosine receptors, cystic fibrosis, and airway hydration. *Handb Exp Pharmacol.* 2009:363–381. [PubMed: 19639288]
42. Shimura S, Sasaki T, Nagaki M, Takishima T, Shirato K. Extracellular ATP regulation of feline tracheal submucosal gland secretion. *Am J Physiol.* 1994; 267:L159–L164. [PubMed: 8074238]

Highlights

- Epithelial loss statistically correlates with TBSA and age
- Epithelial loss did not correlate with time of tissue fixation
- No difference in integrity scores between inhalation and burn only victims
- Loss of the epithelium can promote mucostasis and pneumonia

Figure 1.

A) Micrograph showing a bronchus without an epithelium. The victim had an 88% TBSA, 88% 3rd degree burn injury and died 15 days after injury. Time between death and autopsy (TBDA) was 2 hours. Bar = 400 microns. B) Micrographs showing an obstructed bronchiole from a victim with consistent presence of an epithelium (score 3). The victim had received a

67% TBSA and 3rd degree flame burn. The patient died seven days after injury and the time between death and autopsy (TBDA) was 12 hours. The airway is extensively obstructed and the epithelium partially detached from the mucosal surface. Bar = 200 microns.

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Figure 2.

A. Unadjusted relation between age and bronchiolar epithelial integrity scores with a simple regression line to indicate the trend of lower scores with higher ages. Age is shown on a log scale. Points were randomly jittered horizontally to ensure all were visible. $p = 0.0003$, $R^2 = 0.17$. Open dots indicate scores from burn victims with inhalation injury.

B. Unadjusted relation between percent 3rd degree burn injury and bronchiolar epithelial integrity scores with simple regression line to indicate the trend of lower scores with higher burn percentages. Points were randomly jittered horizontally to ensure all were visible. $p = 0.001$, $R^2 = 0.15$. Open dots indicate scores from burn victims with inhalation injury.

Table 1
Case demographics and issues related to burn injury, ventilatory days, tissue fixation, and pneumonia

Parameter	Percent / Range / Incidence	Mean $\pm$ SD
Age	4 months to 19 years	7.7 $\pm$ 6.1
Gender (males)	53%	NA
Race	78% (H), 17% (C), 5% (B)	NA
TBSA burn	18 to 100%	68 $\pm$ 22%
Extent 3 rd Degree burn	0 to 100%	55 $\pm$ 30%
Ventilatory Days	0 to 88	11 $\pm$ 16
Days After Injury	1 to 240	31 $\pm$ 43
Hours Death to Autopsy	1 to 72	23 $\pm$ 15
Pneumonia	53%	NA
Inhalation Injury	42%	NA

TBSA = total body surface area, SD = standard deviation, H = Hispanic, C = Caucasian, B = Black, NA = not applicable

Table 2

Comparison of the degree of burn injury, days after injury and time between death and autopsy in burn only subjects and burn subjects with inhalation injury.

Condition	Burn Only (n =42)	S+B (n= 30)
TBSA	63 ± 24	75 ± 18 [†]
Percent 3 rd Degree	47 ± 30	65 ± 28 [†]
Days After Injury	31 ± 41	32 ± 46
Hrs. after death to autopsy	25 ± 17	20 ± 10
Ventilatory Days	11 ± 18	11 ± 13
Bronchial Integrity Score	1.6 ± 1.1	1.5 ± 1.1
Bronchiolar Integrity Score	1.8 ± 1.0	1.8 ± 0.9

S+B = smoke inhalation and burn injury,

[†] = statistically different compared to burn only, p = 0.02.

Table 3

Multivariate ordinal logistic regression model coefficient estimates for bronchial and bronchiolar integrity scores, with 95% confidence intervals and p-values. There were no significant effects on bronchial scores.

A: Bronchial Scores				
	Odds Ratio	CI95Min	CI95Max	p-value
log2(Age)	0.76	0.53	1.08	0.13
Third Degree %	0.99	0.97	1.01	0.20
Inhalation Inj.	0.86	0.31	2.36	0.76
Pneumonia	0.49	0.17	1.40	0.19
log2 (Days After Injury)	0.98	0.68	1.41	0.91
log2 (Hours to Autopsy)	0.86	0.58	1.27	0.44
log2 (Ventilation Days)	1.11	0.74	1.66	0.60

B: Bronchiolar Scores				
	Odds Ratio	CI95Min	CI95Max	p-value
log2 (Age)	0.65	0.45	0.93	0.02
Third Degree %	0.98	0.96	1.00	0.02
Inhalation Inj.	1.13	0.45	2.88	0.79
Pneumonia	0.51	0.19	1.34	0.17
log2 (Days After Injury)	0.73	0.51	1.05	0.09
log2 (Hours to Autopsy)	1.13	0.76	1.68	0.56
log2 (Ventilation Days)	1.18	0.80	1.73	0.40