Journal of Critical Care (2013) **28**, 111.e9–111.e15

ELSEVIER

Journal of Critical Care

Expression of acute-phase cytokines, surfactant proteins, and epithelial apoptosis in small airways of human acute respiratory distress syndrome☆,☆☆

Ruy Camargo Pires-Neto PhD[a,*], Maina Maria Barbosa Morales PhD[a], Tatiana Lancas PhD[a], Nicole Inforsato MD[a], Maria Irma Seixas Duarte PhD[a], Marcelo Britto Passos Amato PhD[b], Carlos Roberto Ribeiro de Carvalho PhD[b], Luiz Fernando Ferraz da Silva PhD[a], Thais Mauad PhD[a], Marisa Dolhnikoff PhD[a]

[a]*Department of Pathology, Experimental Air Pollution Laboratory-LIM05, Sao Paulo University Medical School, 01246903 Sao Paulo, Brazil*
[b]*Pulmonary Division, Heart Institute (InCor), Sao Paulo University Medical School, 01246903 Sao Paulo, Brazil*

Keywords:
ARDS;
Respiratory epithelium;
Apoptosis;
Cytokines;
Surfactant protein

Abstract

Purpose: Recent studies suggest a role for distal airway injury in acute respiratory distress syndrome (ARDS). The epithelium lining the small airways secretes a large number of molecules such as surfactant components and inflammatory mediators. There is little information on how these small airway secretory functions are altered in ARDS.

Materials and Methods: We studied the lungs of 31 patients with ARDS (Pao_2/fraction of inspired oxygen ≤ 200, 45 ± 14 years, 16 men) and 11 controls (52 ± 16 years, 7 men) submitted to autopsy and quantified the expression of interleukin (IL) 6, IL-8, surfactant proteins (SP) A and SP-B in the epithelium of small airways using immunohistochemistry and image analysis. In addition, an index of airway epithelial apoptosis was determined by the terminal deoxynucleotidyl transferase-mediated deoxyuridine-triphosphatase nick-end labeling assay, caspase 3, and Fas/Fas ligand expression. The density of inflammatory cells expressing IL-6 and IL-8 within the small airway walls was also quantified.

Results: Acute respiratory distress syndrome airways showed an increase in the epithelial expression of IL-8 ($P = .006$) and an increased density of inflammatory cells expressing IL-6 ($P = .004$) and IL-8 ($P < .001$) compared with controls. There were no differences in SP-A and SP-B epithelium expression or in epithelial apoptosis index between ARDS and controls.

Conclusion: Distal airways are involved in ARDS lung inflammation and show a high expression of proinflammatory interleukins in both airway epithelial and inflammatory cells. Apoptosis may not be a major mechanism of airway epithelial cell death in ARDS.

☆ Supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).
☆☆ The authors have no conflicts of interest to declare.

* Corresponding author. Department of Pathology, Sao Paulo University Medical School, 01246903 Sao Paulo, SP, Brasil.
E-mail address: ruy@usp.br (R.C. Pires-Neto).

0883-9441/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jcrc.2012.05.013

1. Introduction

Airway dysfunction has been increasingly recognized as an important contributor to pulmonary impairment in patients with acute respiratory distress syndrome (ARDS) [1]. Acute respiratory distress syndrome is characterized by the abrupt onset of hypoxemia with diffuse pulmonary infiltrates and an accumulation of a protein-rich pulmonary edema that causes reduction in lung compliance, alveolar collapse, and ventilation-perfusion mismatch [2]. Furthermore, increased lung resistance, expiratory flow limitations, and dynamic hyperinflation have also been reported, which are partially attributed to airway closure [1,3].

Animal models of acute lung injury (ALI) have shown that, in addition to damage to the parenchyma in ALI/ARDS, small airway injuries are characterized by bronchiolar epithelial necrosis and sloughing and by rupture of alveolar-bronchiolar attachments [4-8]. The loss of mechanical alveolar/airway interdependence, airway epithelial injury, interstitial edema, and alveolar collapse may all contribute to distal airway instability [1,7]. We have recently reported that in humans who died with ARDS, small airway changes were characterized by wall thickening with inflammation, extracellular matrix remodeling, and epithelial denudation [9]. Importantly, the degree of airway epithelial denudation in these patients was associated with disease severity.

Previous studies have suggested a role for distal airway epithelium injury in the pathophysiology of human ALI/ARDS [10,11]. The epithelium lining the airways modulates airway function by secreting a large number of molecules such as surfactant components and inflammatory mediators [12]. Changes in the composition and function of the surfactants released by the airways are observed in different pulmonary diseases, such as asthma and chronic bronchitis. Dysfunction of airway surfactants can be associated with the impairment of host defenses and distal airway stability [13]. So far, there is little information on how these secretory functions of the small airways are altered in ARDS. Among the inflammatory mediators involved in lung injury in ARDS, interleukins (IL) 6 and IL-8 were increased in both serum and bronchoalveolar lavage (BAL) [14,15], and the serum interleukins levels were associated with increased mortality and morbidity [14]. The expression of these interleukins in airway epithelial cells has not been addressed in ARDS.

Alveolar cell apoptosis is increased in patients with ARDS and is likely to contribute to alveolar injury; the Fas/Fas ligand (FasL) system, a surface receptor and its natural ligand, seems to play a central role in this process [16]. We have previously reported that bronchiolar epithelium injury and denudation are present in humans with ARDS and are associated with disease severity [9]; the mechanism of airway cell injury in these patients is not known. We hypothesized that apoptosis could be an important mechanism of epithelial cell death not only in the alveolar epithelium but also in the distal airways in ARDS.

In this study, we assessed small airway alterations that could be involved in pulmonary inflammation and surfactant dysfunction in ARDS. For this purpose, we measured airway expression of the inflammatory cytokines IL-6 and IL-8, the airway expression of the surfactant proteins (SP) A and SP-B, and an index of airway epithelial apoptosis of patients with ARDS submitted to autopsy and compared the results with those of control subjects.

2. Methods

This study was approved by the review board for human studies of the São Paulo University Medical School (CAPPesq-FMUSP). The study is retrospective and used archived material from routine autopsies performed at the Autopsy Service of Sao Paulo University Medical School. Consent for performing autopsy was obtained from the next of kin of all the subjects involved in the study.

2.1. Study population

Lung tissue from 31 patients with ARDS submitted to autopsy at Sao Paulo University Medical School between 2004 and 2007 was retrospectively included in the study. Inclusion criteria were a clinical diagnosis of ARDS defined according to the American-European Consensus criteria [17], histologic findings of diffuse alveolar damage [18], an absence of chronic lung diseases, and sufficient archived autopsy material (at least 3 small airways per patient) for analysis. Eleven nonsmoker, nonventilated patients who died of nonpulmonary causes, without previous lung diseases were used as controls. Control subjects showed normal lungs at gross and microscopic examination. We have characterized this population in a previous study [9].

The following clinical data were obtained from medical charts: age, sex, predisposing cause of ARDS, cause of death, days of ARDS evolution (time interval between ARDS diagnosis and death), partial pressure of oxygen (Pa_{O_2}), plateau pressure, positive end-expiratory pressure (PEEP), and the ratio of Pa_{O_2} to fraction of inspired oxygen (F_{IO_2}) assessed at the time of the clinical diagnosis.

2.2. Tissue processing and histologic analysis

Paraffin blocks of lung tissue collected during routine autopsy were retrieved from the archives of the Department of Pathology of Sao Paulo University Medical School. In the routine autopsies, 3 to 4 fragments of lung tissue were collected from any regions of altered lung parenchyma. In normal lungs, 1 fragment of lung tissue was collected from each lobe. The tissue had been previously fixed in 10% buffered formalin for 24 hours, routinely processed and paraffin embedded. Sections 5 μm thick were stained with hematoxylin and eosin for histologic diagnoses of