CHEST Original Research

BIOMARKERS

Proteomic Analysis of Undiluted Lung Epithelial Lining Fluid*

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Background: **Proteomics is increasingly leading to biomarker discovery in human disease. Epithelial lining fluid (ELF), until now only recovered indirectly, diluted in BAL fluid, is an attractive sample for lung disease proteomics. The direct recovery of undiluted ELF is now possible using a bronchoscopic microsampling (BMS) probe. In this preliminary study of anesthetized ventilated rabbits, we applied this probe to recover ELF and to analyze the resulting samples with the aim of determining their potential in lung disease biomarker discovery.**

Methods: **In order to do so, a method was devised and evaluated in preliminary experiments both for nonbronchoscopic use of the probe and for recovering undiluted ELF from probe tips. To verify the proteomic potential of the sample, the recovered ELF was separated by one-dimensional polyacrylamide gel electrophoresis, and the resulting lane was cut into multiple fractions, each of which was digested and analyzed by liquid chromatography tandem mass spectrometry. The identified proteins were then searched against Medline for association with broad categories of lung disease.**

Results: **Nonbronchoscopic use of the probe allowed successful ELF sampling and the recovery of undiluted ELF from probe tips. Proteomic analysis showed that ELF contains many proteins that have already been reported as being associated with lung disease as well as proteins potentially correlated with lung disease.**

Conclusions: **This preliminary study of undiluted ELF, as recovered by the BMS probe, shows that it may be an ideal sample for lung proteomics. The potential application of this sampling technique in various lung diseases will need to be confirmed by future studies.** ***(CHEST 2008; 134:338–345)***

Key words: biomarker; lung disease; mass spectrometry; proteome

Abbreviations: ALI = acute lung injury; BALF = BAL fluid; BMS = bronchoscopic microsampling; ELF = epithelial lining fluid; FDR = false discovery rate; LC = liquid chromatography; MS/MS = tandem mass spectrometry; 1D-PAGE = one-dimensional polyacrylamide gel electrophoresis

Epithelial lining fluid (ELF), the water, electrolyte, and biomolecule content lining the respiratory epithelium, is an attractive sample for the study of lung physiology or pathogenesis.[1,2] The indirect analysis of ELF, by sampling BAL fluid (BALF), has long been a mainstay of clinical and experimental investigation of lung disease as the composition of BALF has been considered to reflect that of ELF. This paradigm of BALF in lieu of ELF has carried over into the proteomic search for lung disease biomarkers,[3] which has exclusively focused on BALF.[4–14] However, BAL dilutes ELF variably up to 130-fold,[15] which leads to erroneous estimations of ELF volume and content[16,17] even when using dilution markers such as urea. This inaccuracy is furthered by the increased alveolar capillary permeability induced by lavaging the lung.[18] The BAL procedure, consisting of an instillation of saline solution, interferes with proteomic analysis by increasing the salt content of the sample.[3,6] Thus, variably diluted ELF, as recovered in the form of BALF, cannot be considered an ideal sample for proteomic analysis, whereas undiluted ELF might be promising.[1]

In order to recover undiluted ELF samples, Ishizaka et al[19] developed a bronchoscopic microsampling (BMS) probe. However, in all of the studies using this probe,[19–22] ELF was recovered by probe tip dilution in saline solution, which would interfere with proteomic analysis.[3,6] In order to simplify the proteomic analysis of ELF, a prerequisite of our study was to devise a new means to recover undi-

luted ELF from the probe tips. This was achieved through tip suspension and centrifugation, and was verified in preliminary experiments in anesthetized rats. Another objective was to devise a nonbronchoscopic means of using the probe that would allow the use of adult probes in a small animal such as rabbits while still being relevant for use in humans.

Our main hypothesis was that undiluted ELF thus recovered could be successfully applied to proteomic analysis. Additionally, we wished to determine whether the identified proteins were relevant to lung disease pathophysiology. We conducted a preliminary, proof-of-concept experimental study in anesthetized, ventilated rabbits to determine the feasibility of using the BMS probe nonbronchoscopically to recover undiluted ELF, and, subsequently, of analyzing its proteomic content using liquid chromatography (LC), tandem mass spectrometry (MS/MS) after one-dimensional polyacrylamide gel electrophoresis (1D-PAGE) separation.

Materials and Methods

Animal Investigation Protocol

Animal experimental protocols were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the University of California–San Francisco Local Animal Research Committee requirements. Certified pathogen-free male New Zealand white rabbits (3.6 to 4.4 kg; Western Oregon Rabbit; Philomath, OR) were used.

Animal Preparation and General Protocol

Following the subcutaneous administration of ketamine (30 mg/kg) allowing IV line placement, anesthesia was induced IV with sodium pentobarbital (30 mg/kg) and was maintained with halothane (1%). After tracheotomy, a 4-mm-diameter endotracheal tube was inserted. Mechanical ventilation was maintained with a constant-volume pump (Harvard Apparatus Inc; Holliston, MA), with a fraction of inspired oxygen of 1.0 at a tidal volume of 20 mL/kg body weight; a positive end-expiratory pressure of 3 cm H_2O was applied.

Nonbronchoscopic Use of the BMS Probe and ELF Sampling Procedure

The BMS probe (BC-401C; Olympus Co; Tokyo, Japan) and bronchoscopic sampling procedure have previously been described in detail.[19] Given the small diameter of rabbit airways, we had to proceed nonbronchoscopically. In order to do so, we modified the existing bronchoscopic protocol by introducing the BMS probe through the outer sheath of a blind protected distal catheter (Combicath; Plastimed; Le-Plessy-Bouchard, France) instead of through a bronchoscope (Fig 1). The procedure was repeated three times per animal. Because the recovered samples were destined for proteomic analysis requiring only minute amounts, we limited the number of animals to three. The procedure is highlighted in an online animated supplement.

Recovery of Undiluted ELF From the BMS Probe

For proteomic analysis, we developed a novel centrifugation technique to recover undiluted ELF from the tip. The catheter wire was cut 2 to 3 cm above the hilt and was suspended in a centrifuge tube so that the fiber tip was not in contact with the

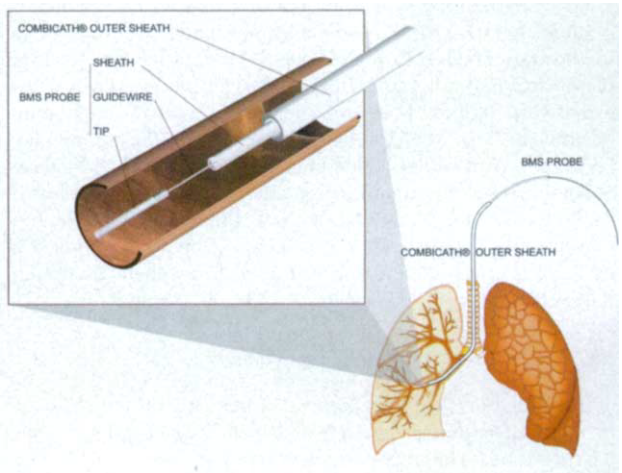

FIGURE 1. Modified, nonbronchoscopic ELF sampling procedure. Briefly, following anesthesia, tracheotomy and intubation, the outer sheath of a Combicath catheter is inserted in the trachea, and advanced until slight resistance is observed. The sheathed BMS probe is then inserted through the outer sheath of a Combicath. The outer sheath of the BMS probe is advanced until the protective plug of the Combicath is ejected, and then further until slight wedging of the sheath in a distal bronchus. While holding the outer sheath of the probe at the target in the subsegmental bronchus, the inner wire-mounted tip is slowly advanced toward distal respiratory epithelium until slight resistance is observed indicating contact. The probe tip is maintained in position for 10 s allowing the fiber tip to absorb lung ELF. The inner probe is then withdrawn into its outer sheath to avoid contamination, and out of the Combicath, which is then itself withdrawn.

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