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Original Article

Iron accumulates in the lavage and explanted lungs of cystic fibrosis patients

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Abstract

Oxidative stress participates in the pathophysiology of cystic fibrosis (CF). An underlying disruption in iron homeostasis can frequently be demonstrated in injuries and diseases associated with an oxidative stress. We tested the hypothesis that iron accumulation and altered expression of iron-related proteins could be demonstrated in both the bronchoalveolar lavage (BAL) fluid and explanted lungs of patients with cystic fibrosis. BAL fluid collected from 10 children with CF showed elevated concentrations of protein, iron, ferritin, transferrin, heme, and hemoglobin relative to that obtained from 20 healthy volunteers. Using Perl's Prussian blue staining, explanted lung from CF patients revealed increased iron in alveolar and interstitial macrophages. Similarly, there was an increased expression of ferritin, the iron importer DMT1, and the exporter ferroportin 1 in lung tissue from CF patients. We conclude that iron homeostasis is disrupted in CF patients with an accumulation of this metal and altered expression of iron-related proteins being evident in the lungs.
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Keywords: Iron; Ferritin; Cystic fibrosis; Transferrin; Lung diseases

1. Introduction

The relationships between the presence of a CFTR mutation, cell dysfunction, tissue injury, and the variety of clinical manifestations of cystic fibrosis are not well understood. A current postulate suggests that abnormal ion transport through the CFTR protein leads to paradoxical changes in sodium uptake, dehydrated lining fluid, and a collection of viscous mucus [1]. This mechanistic theory supports a pathway of injury in CF which focuses on respiratory and gastrointestinal obstruction by thickened secretions leading to infection and parenchymal remodeling.

Oxidative stress, resulting from both an increased generation of reactive oxygen species (ROS) by host cells and decreased anti-oxidant concentrations, is believed to participate in the pathophysiology of CF [2,3]. Evidence of such oxidative stress in CF can be observed in elevated measures of plasma malondialdehyde (MDA) levels [4,5] and 8-isoprostane [4,6] as well as breath pentane and ethane [7]. Factors which contribute to an increased production of ROS in CF patients include an airway inflammation, that can precede the introduction of microbes into the respiratory tract [8,9], and recurrent infections which are associated with a further inflammatory influx that produces high levels of ROS [10]. In addition, individuals with CF can demonstrate an elevated metabolic rate associated with an increase of ROS (superoxide and hydrogen peroxide) continuously generated as mitochondrial products of the respiration process [11]. Regarding anti-oxidants, investigation revealed low levels of plasma antioxidant vitamins in CF patients with malabsorption contributing to a decreased uptake of fat soluble

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vitamin E and beta-carotene [3,4]. Lower concentrations of the anti-oxidant glutathione have been quantified in the epithelial lining fluid and serum of CF patients relative to control subjects [12]. One of the postulated reasons for deficiency of this specific anti-oxidant is a loss of permeability in the CFTR channel for glutathione [13,14]. Accordingly, there are several interacting networks which can produce an increased oxidative stress in CF patient.

In numerous injuries and diseases associated with an oxidative stress, an underlying disruption in iron homeostasis can frequently be demonstrated [15]. There is evidence to support such an accumulation of iron in the respiratory tract of individuals diagnosed to have CF [16–18]. We tested the hypothesis that iron accumulation and altered expression of iron-related proteins can be demonstrated in the lavage and explanted lungs of CF patients.

2. Materials and methods

2.1. Bronchoalveolar lavage of patients and healthy volunteers

Bronchoalveolar lavage fluid was obtained from ten children with CF undergoing clinically indicated bronchoscopy. Details of bronchoscopy have been previously described [19,20]. Patients were anesthetized with inhaled sevoflurane and intravenous propofol. Lidocaine was applied to the vocal cords and carina for topical anesthesia. Generally, lavage was directed at areas of either radiographic abnormality or visibly heavy secretion. For each lavage, 1 mL/kg aliquots (up to a maximum of 20 mL) of normal saline were instilled and suctioned back through the bronchoscope. Parents and, as appropriate, patients gave consent to use of the BAL fluid for research based on an IRB-approved protocol.

Twenty healthy non-smoking volunteers (18 to 40 years of age) also underwent fiberoptic bronchoscopy with bronchoalveolar lavage (BAL). Lidocaine only was employed in anesthesia for healthy volunteers [21]. The fiberoptic bronchoscope was wedged into a segmental bronchus of the lingula and then the right middle lobe. Fifty mL aliquots of sterile saline were instilled and immediately aspirated (to a total of 250 mL), centrifuged, and stored at −70 °C. Volunteers consented to the protocol which had been approved by the University of North Carolina School of Medicine Committee on the Protection of the Rights of Human Subjects.

Prior to assays of human lavage samples, comparability was confirmed by quantifying urea nitrogen concentrations (Thermo Electron, Louisville, CO).

2.2. Protein, iron, ferritin, and transferrin concentrations in the BAL fluid

Lavage protein levels were employed as an index of lower respiratory injury; these were determined using the Pierce Coomassie Plus Protein Assay Reagent (Pierce, Rockford, IL). Lavage iron was measured using a colorimetric, enzymatic method (Sigma Diagnostics, St. Louis, MO). Ferritin concentrations were measured using an enzyme immunoassay (Microgenics Corporation, Concord, CA). Transferrin concentrations were analyzed using an immunoprecipitin analysis (INCSTAR Corporation, Stillwater, MN).

2.3. Heme and hemoglobin concentrations in the BAL fluid

Heme and hemoglobin in the lavage were quantified using colorimetric methodology (QuantiChrom Heme and Hemoglobin Assay Kits; BioAssay Systems, Hayward, CA).

2.4. Interleukin (IL)-8 levels in BAL fluid

Lavage fluid was analyzed using ELISA (R&D Systems, Minneapolis, MN) for interleukin (IL)-8, a pro-inflammatory mediator whose release strongly correlates with oxidative stress [22–25].

2.5. Histochemistry and immunohistochemisty

Staining was done on six lungs resected from CF patients receiving transplantation. An equal number of control specimens were obtained from lung peripheral to neoplasm in patients having pneumonectomy for resection of lung cancer. All tissues were freshly obtained. Use of these tissues was deemed exempt from consent per our institutional IRB.

Tissue sections were cut, floated on a protein-free water bath, mounted on silane treated slides (Fisher, Raleigh, NC), and air-dried overnight. Sections were stained for iron using Perl's Prussian Blue. Immunohistochemistry for ferritin was performed using a human anti-ferritin antibody (Dako, Carpinteria, CA) at a dilution of 1:100 [26]. In addition, primary antibodies to divalent metal transport 1 (DMT1) (generously provided by Dr. Funmei Yang of the University of Texas, San Antonio, TX) and ferroportin 1 (FPN1) (generously provided by Dr. David Haile of the University of Texas, San Antonio, TX) were used at a dilution of 1:200 [27,28]. Slides were then incubated with biotinylated linking antibody from Stat-Q Staining System (Innovex Biosciences, Richmond, CA) for 10 min at room temperature, washed with PBS, and applied peroxidase enzyme label from Stat-Q Staining System (Innovex Biosciences). After incubation for 10 min at room temperature and washes with PBS, tissue sections were developed with 3,3′-diaminobenzidine-tetrahydrochloride for 3 min at room temperature. Sections were counterstained with hematoxylin, dehydrated through alcohols, cleared in xylene and coverslipped using a permanent mounting media.

2.6. Statistics

Data are expressed as mean value ± standard error unless otherwise specified. T tests of independent means were used to compare lavage results from the healthy volunteers and patients with cystic fibrosis. Two-tailed tests of significance were employed. Significance was assumed at $P < 0.05$.

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