SHORT COMMUNICATION

Lung hyaluronan levels are decreased in alpha-1 antiprotease deficiency COPD

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Hyaluronan; Lung; Elastic fibers; COPD; Alpha-1 antiprotease deficiency

Summary

Introduction: Hyaluronan (HA), a long-chain polysaccharide, is currently being evaluated as a potential therapeutic agent for pulmonary emphysema, based on previous studies from this laboratory indicating its protective effect against elastic fiber breakdown. To determine whether exogenously administered HA might replace a loss of this extracellular matrix component in this disease, we measured the content of HA in lung biopsies from both healthy individuals and alpha-1 antiprotease-deficient (AAPD) COPD patients with pulmonary emphysema.

Methods: Tissue samples (9 from COPD patients, 5 from controls) were digested with papain to isolate glycosaminoglycans, and lung HA was quantified with an enzyme-linked immunosorbent assay.

Results: HA was significantly decreased in the AAPD-COPD population compared to normal individuals (13.5 vs 21.7 ng/mg wet lung; p < 0.01). Furthermore, there was a positive correlation between HA levels and the following parameters: 1) percent predicted FEV1 (r = 0.78; p < 0.01), 2) percent predicted DLCO (r = 0.74; p < 0.05), and 3) serum levels of AAP (r = 0.61; p < 0.05).

Conclusions: These findings support the hypothesis that depletion of lung HA plays a role in the pathogenesis of pulmonary emphysema, and that replacement of this matrix component could slow the progression of the disease.

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Introduction

Chronic obstructive pulmonary disease (COPD) involves progressive damage to the elastic fiber network of the lung, resulting in dilatation and rupture of alveoli [1,2]. While most approaches to treating COPD have focused on the use of elastase inhibitors to reduce elastic fiber damage and loss of alveoli, our laboratory is developing a novel therapy involving inhalation of aerosolized hyaluronan (HA). The rationale for this treatment is based on studies indicating that nebulized HA prevents elastic fiber breakdown and significantly reduces airspace enlargement in experimental models of pulmonary emphysema induced by either intratracheal instillation of elastase, or exposure to cigarette smoke [3–8].

While these findings provide a basis for testing the effects of exogenously administered HA in COPD patients with pulmonary emphysema, this approach would become more compelling if their lungs were shown to be depleted of this matrix component. To date, the relationship between HA content and emphysematous changes has not been adequately investigated. Several previous reports indicate that HA is decreased in either intact lungs or airway smooth muscle cells from patients with COPD, but none of these studies correlated the degree of depletion with parameters of pulmonary function [9–11].

The current study addresses this issue by measuring HA in lung biopsies from both alpha-1 antiprotease-deficient (AAPD) COPD patients and a control group that have undergone extensive evaluation for pulmonary disease. The results indicate that the level of HA is significantly reduced in the COPD population, and correlates with: 1) forced expiratory volume at one second (FEV₁), 2) diffusing capacity of the lung for carbon monoxide (DLCO), a measure of lung surface area, and 3) serum levels of AAP. These findings further support the hypothesis that reductions in lung HA content contribute to the development of pulmonary emphysema, and that supplementation therapy may slow the progression of the disease.

Methods

Procurement of lung tissue

Lung parenchymal biopsies (200–350 mg each) from 9 AAPD COPD patients and 5 control individuals were obtained from the Lung Tissue Research Consortium (Owings Mills, MD), which is conducting a long-term study of COPD, approved and sponsored by the NIH Clinical Center. Tissues were procured during lung volume reduction surgery, resections for tumors, or lung transplantation. All specimens were reviewed by a pathologist to confirm the presence or absence of disease. However, the individual biopsies derived from this tissue were not separately examined.

Subjects involved in the investigation signed an informed consent document, and underwent comprehensive testing for pulmonary disease, including CT scans and lung function studies. Clinical data gathered by the consortium were coded and made available for the current investigation, which was approved by the institutional review board at St Luke’s-Roosevelt Hospital Center.

Physiologic measurements

The forced expiratory volume measurements were performed by recording the amount of air leaving the lungs during maximum-effort exhalation. The volume associated with the first second of this process (FEV₁) was converted to a percent predicted value, based on anthropometric data from a healthy population.

DLCO testing involved measuring the amount of carbon monoxide (CO) absorbed by the lungs over a specified interval. Following inhalation and holding of the test gas for 10 s, a sample of the exhaled air was measured for CO, and the results were converted to a percent predicted value, based on data from an appropriate reference population.

Separation of glycosaminoglycans

Lung tissues were weighed, washed in normal saline, and incubated at 65 °C for 20 h with papain (Sigma–Aldrich, St Louis, MO), dissolved in 0.15 M phosphate buffer, pH 6.2, containing 0.01 M cysteine and 0.01 M EDTA. Following digestion, the samples were centrifuged, and the supernatants were analyzed for HA content, as described below.

Measurement of HA

The content of HA in the solubilized fraction of the lung samples was determined by an immunoassay involving competitive binding of the polysaccharide to a specially prepared detector protein (Echelon Biosciences, Salt Lake City, UT). Samples were mixed with the protein, and added to a 96-well microplate coated with HA. An enzyme-linked antibody was then used to determine the amount of detector bound to the plate (alkaline phosphatase/p-nitrophenyl phosphate substrate). The colorimetric signal was read at 405 nm and compared to a standard curve. Results were expressed as ng HA per mg wet lung, and represent an average of two separate determinations performed in triplicate.

Statistical analysis

The two-tailed t-test was used to determine statistically significant differences (p < 0.05) between COPD patients and normal individuals. Linear regression analysis, including calculation of the Pearson correlation coefficient (r), was used to correlate HA content with FEV₁, DLCO, and serum AAP levels.

Results

Patient population

COPD patients had an age range of 44–64 years of age, with a mean of 56, whereas control ages varied from 45 to 81, with a mean of 64. Eight of the nine COPD patients and one of the five controls had a documented smoking history, but none were current smokers. All members of both groups were Caucasian.
The COPD group had significantly lower mean serum AAP levels compared to controls (71 vs 148 mg/dl; \( p < 0.0001 \)), and mean percent predicted FEV\(_1\) was also significantly lower (34 vs 94; \( p < 0.001 \)). However, there was no significant difference between the COPD patients and controls with regard to percent predicted DLCO (69 vs 86, respectively; \( p > 0.05 \)).

**HA measurements**

Lung tissues derived from the COPD and control populations were measured for HA content, and the results were normalized to the wet weight of the samples. The mean level of lung HA was significantly lower in COPD patients compared to controls (13.5 vs 21.7 ng/mg; \( p < 0.01 \); Fig. 1). As also shown in Fig. 1, there were positive correlations between lung HA content and the following parameters: 1) percent predicted FEV\(_1\) (\( r = 0.78; p < 0.001 \)), 2) percent predicted DLCO (\( r = 0.74; p < 0.05 \)), and 3) serum levels of AAP (\( r = 0.61; p < 0.05 \)).

There was no significant difference in HA levels between patients with CT-scan diagnoses of either panlobular or centrilobular emphysema (10.9 vs 13.6 ng/mg, respectively). However, one AAPD subject with no CT evidence of emphysema had an HA level of 23.7 ng/mg, well within the control range.

**Discussion**

The concept of using HA to treat pulmonary emphysema is based on a series of experiments designed to determine whether agents other than elastases were capable of inducing pulmonary emphysema. A nonelastolytic enzyme, hyaluronidase, was shown to produce pulmonary airspace enlargement in hamsters when administered in conjunction with 60 percent oxygen [12]. Damage to elastic fibers occurred only when both agents were given concomitantly, suggesting that hyaluronidase may facilitate the breakdown of these fibers by making them more accessible to injury. This hypothesis was supported by subsequent studies demonstrating that pretreatment of the lung with hyaluronidase enhances elastase-induced airspace enlargement [6].

Experiments were then undertaken to examine the effect of HA itself on this model of emphysema. Animals treated with aerosolized HA prior to instillation of elastase showed significantly less airspace enlargement than controls treated with elastase alone [3–6]. The adherence of HA to elastic fibers suggested that it may protect them from enzymatic breakdown, acting as a physical barrier to cells and enzymes responsible for elastolysis [3,5]. Such protection may also occur naturally, because HA has been shown to be in close anatomical proximity to elastic fibers [13]. Removal of HA from the periphery of damaged fibers, due to unchecked neutrophil elastase activity, could therefore account for the observed correlation between HA and AAP levels.

HA may also improve the mechanical properties of elastic fibers by virtue of its ability to retain water [14]. The absorption of water onto nonpolar hydrophobic groups during the extension of elastic fibers contributes to the storage of elastic energy [15,16]. A decrease in the availability of water can compromise this process, reducing elastic fiber recoil. Evidence for this hypothesis was provided by a recent study indicating that HA and other proteoglycans help to stabilize alveolar walls by reducing the uneven distribution of forces in the extracellular matrix [17]. This finding suggests that loss of HA might increase the likelihood of alveolar wall rupture, thereby trapping air in the lung and reducing gas exchange surface area, which, in turn, would adversely affect FEV\(_1\) and DLCO.

The generally slow progression of pulmonary emphysema suggests that even a small decrease in the rate of alveolar wall injury could have an important impact on the disease process. By protecting elastic fibers from enzymatic and mechanical damage, the use of aerosolized HA could potentially alter the natural history of this disorder, thereby reducing the risk of respiratory failure.

**Conflict of interest**

None of the authors involved in this study have a conflict of interest with regard to the work.
References


