CHEST

The Therapeutic Potential of Hyaluronan in COPD

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Insights into the clinical course of COPD indicate the need for new therapies for this condition. The discovery of alpha-1 antitrypsin deficiency (AATD) led to the protease-antiprotease imbalance hypothesis, which was applied to COPD related to AATD as well as COPD not related to AATD. The discovery of AATD brought recognition to the importance of elastin fibers in maintaining lung matrix structure. Two cross-linking amino acids, desmosine and isodesmosine (DI), are unique to mature elastin and can serve as biomarkers of the degradation of elastin. The intravenous augmentation treatment and lung density in severe alpha-1 antitrypsin deficiency (RAPID) study shows a correlation of an anatomic index of COPD (on CT imaging) correlating with a chemical indicator of matrix injury in COPD, DI. The results suggest that preservation of lung elastin structure may slow the progression of COPD. Hyaluronan aerosol decreases the severity of elastase-induced emphysema in animals and has induced reductions in DI levels in preliminary human studies. Hyaluronan deserves further development as a therapy for COPD. CHEST 2018; 153(4):792-798

KEY WORDS: COPD; desmosine; elastin; emphysema; hyaluronan

Recent reports, added to past insights into the clinical course of COPD, indicate the need for new therapies for this condition.[1,2] The discovery of alpha-1 antitrypsin deficiency (AATD)[3] led to the protease-antiprotease imbalance hypothesis, which was applied to COPD related to and COPD not related to AATD,[4] the latter involving smoke-related loss of inhibitory capacity of alpha-1 protein for elastases.[5] The discovery of AATD brought recognition to the importance of elastin fibers in maintaining lung matrix structure.[6] Recognition of elastin as a vulnerable structural component in COPD and early insights into the chemical structure of elastin led to the investigation of two cross-linking amino acids that are unique to mature elastin—desmosine and isodesmosine (DI), which can serve as biomarkers of the degradation of elastin in the body matrix and specifically the lung.[6,7] Since the studies, which demonstrate the therapeutic potential of hyaluranon (HA) in AATD COPD and non-AATD COPD, are related to the use of biomarkers of elastin degradation, a discussion of the development of such biomarkers and their application as therapeutic end points follows.

Elastin Biomarker Development

Early studies using the analytic methods of radioimmunoassay and enzyme-linked

ABBREVIATIONS: AATD = alpha-1 antitrypsin deficiency; DI = desmosine and isodesmosine; HA = hyaluronan

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immunoassay found correlations with the clinical diagnosis of COPD and levels of desmosine and isodesmosine (DI), despite limited specificity and sensitivity.[7] It was demonstrated that smokers without COPD and patients with COPD excreted more desmosine in urine than did healthy never smokers.[8]

Smokers with a more rapid lung function decline in a normative aging study had higher levels of desmosine excretion than did those with a slow decline in lung function.[9] Patients with COPD and exacerbations excreted more desmosine than did patients with stable COPD.[10]

However, subsequent studies have not demonstrated consistent elevations of urinary levels of DI in patients with COPD. In an early study using liquid chromatography and mass spectrometry for DI analysis, elevation of urinary levels of DI could not be demonstrated in patients with COPD related to AATD and COPD not related to AATD. That study showed a significant increase in the percentage of free DI in the total DI excretion, possibly a manifestation of the increased inflammatory state in COPD.[11] Ongay et al[12] studied urinary excretion of DI in 365 patients with COPD in the Evaluation of COPD Longitudinally to Identify Predictive Surrogate End Points (ECLIPSE) study using liquid chromatography and tandem mass spectrometry.[12] They reported that age, sex, BMI, and smoking have a significant effect on urinary excretion of DI and should be corrected for in analysis. However, they demonstrated a statistically significant increase in DI between individuals with stable COPD and healthy control subjects. Huang et al,[13] in a study of 390 patients with COPD, found an increase in urinary DI levels only during exacerbations. Conversely, Boutin et al[14] found a statistically significant decline in urinary DI levels in patients with COPD and a rapid decline in lung function when compared with nonsmoking healthy control subjects. The basis of these differing results is unclear; however, levels of DI in urine are dependent on kidney function so that a short collection period or spot urine samples may be susceptible to greater variability.

It is noteworthy that in the development of biomarkers of elastin degradation, there was early recognition of the antigenicity of elastin peptides produced by enzymatic digestion of human lung elastin.[15] In this regard, Skjot-Arkil et al[16] used matrix metallopeptidases 9 and 12 to develop an elastin peptide antigen for an enzyme-linked immunoassay to measure elastin peptides in the serum of patients with COPD. Marked elevation of such peptides was detected.

Schriver et al[17] also demonstrated statistically significant increases in elastin peptides in urine, plasma, and BAL fluid in patients with COPD. However, such peptides are derived from tropoelastin, the precursor of mature elastin, as well as mature elastin, and may thus represent both breakdown and resynthesis of this protein.

As indicated earlier, analytic methods for DI measurement depended on the development of antibodies to DI, which led to variable results from uncertain antibody specificity. This led to variability in quantification in published studies.[7] In 2003, our laboratory introduced a new methodology into biomarker studies using DI: liquid chromatography and tandem mass spectrometry[11] and advances using a deuterium standard.[18]

This method has been applied to a number of studies that correlate with the presence and severity of COPD and the response to therapies. Studies have shown increased levels of DI in plasma, urine, and sputum in COPD and AATD COPD and have demonstrated that AATD COPD has higher levels than non-AATD COPD.[19] A 2-month study of tiotropium aerosol therapy demonstrated lowered DI levels in plasma, urine, and sputum in patients with COPD.[20] DI levels have also been shown to be increased in plasma in patients exposed to second-hand smoke.[21]

Using this biomarker, two studies for the first time demonstrated the response of DI to augmentation therapy in AATD. DI was statistically significantly reduced over a 6-week period in plasma and BAL fluid with both IV and AAT protein replacement by aerosol.[22] This study was followed by the intravenous augmentation treatment and lung density in severe alpha-1 antitrypsin deficiency (RAPID) study, which recorded statistically significant preservation of lung density over a 4-year study of patients with AATD receiving augmentation therapy compared with an increased loss of lung density in patients receiving placebo.[23] Levels of DI in plasma in patients studied over 4 years showed reductions in elastin degradation as early as 3 months after initiating augmentation therapy and continuing over 4 years of therapy.[24]

Significance of the Results of the RAPID Study

The RAPID study shows, for the first time, a correlation of an anatomic index of COPD (on CT imaging) correlating with a chemical indicator of matrix injury in COPD (DI). The results suggest that preservation of lung elastin structure may slow the progression of

COPD from its beginning. Notably, augmentation therapy preserves elastin by inhibiting, specifically, neutrophil elastase. However optimal reduction of elastin degradation in COPD will require agents that can block the action of metalloproteases and other proteases that participate in the inflammatory process. However, results thus far offer evidence to justify the development of therapies that preserve lung elastin and that can be initiated as therapy at the earliest evidence of COPD to alter the natural history of COPD.

Glycosaminoglycans and Elastin

The RAPID study suggests a therapeutic role for agents that can preserve lung elastin structure and function in COPD. In this regard, it is relevant to recognize the structural and functional roles of matrix constituents that interact with mechanical load-bearing elements such as elastin and collagen. Regarding this, proteoglycans are prominent constituents of the matrix that have been shown to impose stiffness on alveolar structure and have strong structural interactions with collagen and elastin in situ in animal models.[25] Among the matrix molecules, glycosaminoglycans and especially hyaluronan (also called hyaluronic acid) have special relevance regarding the vulnerability of matrix elastin to the effects of elastases.[26,27]

HA is a naturally occurring linear polysaccharide with repeating disaccharide units composed of glucuronic acid and N-acetyl glucosamine. HA is part of a category of structurally similar polysaccharides called glycosaminoglycans, which includes chondroitin sulfate, dermatan sulfate, keratan sulfate, heparin sulfate, and heparin. HA differs structurally from other glycosaminoglycans by the absence of sulphate groups. HA has a unique structural and functional characteristic in its hydration. HA has a coiled configuration in which the water content is approximately 1,000 times greater than the polymer mass. HA functions as a major matrix constituent in which cells and fibrous components such as elastin and collagen are embedded. HA is structurally embedded in the elastic fiber and the microfibrils of collagen in its hydrated state and could provide a barrier function against degradation by proteases.[27]

The concept of using HA to treat pulmonary emphysema emerged from the observation that a nonelastolytic enzyme, hyaluronidase, could produce pulmonary airspace enlargement in hamsters when administered in conjunction with 60% oxygen.[28] Damage to elastic fibers occurred only if both agents were given concomitantly, suggesting that hyaluronidase might make the fibers more accessible to injury. This hypothesis gained further support in subsequent studies indicating that hyaluronidase enhances elastase-induced airspace enlargement.[29]

These findings prompted another series of experiments designed to determine whether exogenously administered HA could have a protective effect in the elastase model of emphysema. These studies demonstrated that aerosolized HA significantly reduced elastase-induced airspace enlargement.[30-32] The subsequent observation that HA adheres to elastic fibers suggested that it protects them from enzymatic breakdown, acting as a barrier to agents responsible for elastolysis.[31-33] This process may also occur naturally, since HA has a close anatomic association with elastic fibers.[34]

HA could also improve the mechanical properties of elastic fibers by virtue of its ability to retain water.[35] Absorption of water onto nonpolar hydrophobic groups when elastic fibers are stretched contributes to the storage of energy.[35,36] A loss of water would compromise this process, reducing elastic fiber recoil. Recent support for this hypothesis was provided by a study demonstrating that HA and other proteoglycans stabilize alveolar walls by reducing uneven distribution of forces in the extracellular matrix.[37] Decreased hydration of elastic fibers might also reduce their structural integrity, making them susceptible to mechanical breakdown. This concept is supported by morphologic studies demonstrating fragmentation of these fibers in human lungs with emphysema.[38]

The protective role of HA against elastin degradation was further demonstrated by in vitro studies using carbon-14-labeled elastin fiber matrices produced by rat mesothelium,[39] which synthesizes a rich elastin fiber matrix. Subsequent to these studies, the protective role of HA aerosol was demonstrated in mice chronically exposed to tobacco smoke over a period of 6 months.[40] We hypothesized that aerosolized HA inhaled daily could protect against lung elastic fiber injury and subsequent destruction of alveoli. Daily treatment with aerosolized HA significantly reduced the severity of emphysema in mice exposed daily to tobacco smoke. The study also found no evidence that HA elicits an immune response or causes pulmonary toxicity.[40]

Mean linear intercept, a measure of the structural integrity of the alveoli, served as the primary quantitative indicator of disease progression. The larger the increase in mean linear intercept, the greater the

damage. Tobacco smoke-exposed mice treated with aerosolized water only (control animals) experienced substantial airspace enlargement by the second month of the study. Smoke-exposed mice treated with aerosolized HA sustained 43% less airspace enlargement over the 6-month study. The protective effect of treatment was statistically significant ($P < .0001$).[40] A comparable margin of protection in humans would hold notable clinical significance.

Other studies have indicated the pathologic effect of exposure to tobacco smoke on the HA content of the lung. In vitro studies of HA exposed to tobacco smoke have indicated the degradation of HA by tobacco smoke exposure.[41] Also, animal lungs exposed to tobacco smoke have diminished HA content.[42] In humans, analysis of glycosaminoglycans in patients dying of COPD has shown a 50% reduction in HA content in the lung parenchyma.[43] This is the only glycosaminoglycan among those including heparan sulfate, chondroitin sulfate, and dermatan sulfate that was found to be reduced.[43] Similar reductions in HA content are present in postmortem lungs of patients with AATD.[44]

The exact mechanism by which HA protects elastin is not yet well understood. We know that HA does not chemically inhibit elastases. Instead, HA appears to bind to elastic fibers and prevents elastases from accessing and attacking the elastic fiber surface, perhaps by surrounding elastic fibers with a molecular network that forms a physical barrier to elastase action (Fig 1).[45-48] HA self-aggregates, and exogenously administered HA may combine with endogenous HA to form larger molecular complexes.[45]

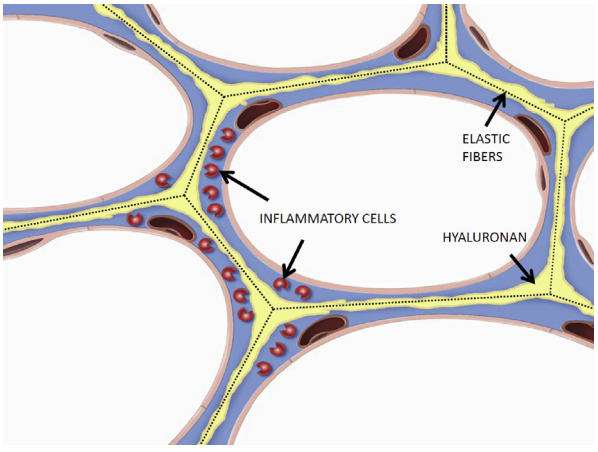

Figure 1 – *The role of hyaluronan as a functional barrier for elastic fibers in alveoli.*

Early Clinical Studies of HA

Two prior studies in human subjects indicated (1) a tolerance of 20 normal subjects to single doses of HA aerosol 1 week apart that had no significant effects on spirometric or routine laboratory values and (2) single doses of HA aerosol to patients with ATTD-related pulmonary emphysema demonstrated no adverse effects on spirometric or laboratory results or tolerance to the administration of the aerosol.[49,50]

Thus far, studies of asthma in another laboratory in Europe demonstrated a protective effect of HA aerosol in exercise-induced asthma and methacholine-induced bronchoconstriction, which indicates the possible therapeutic role of HA in asthma as well as COPD.[51,52]

One of the therapeutic virtues of HA aerosol is that it has not demonstrated any toxic manifestations and therefore could be prescribed at the earliest indications of the development of COPD, which could slow or prevent the progression of lung injury leading to disabling respiratory failure over subsequent years. No such therapy exists at present for such a protective effect, which can affect the natural history of COPD.

Two studies are worthy of mention regarding the future development of HA in COPD: (1) A study in mice demonstrated that the delayed administration of HA aerosol to tobacco smoke-exposed mice (which more closely mimics the potential use of HA in humans) 1 month after the tobacco smoke injury was begun was still effective in decreasing the progression of tobacco smoke-induced emphysema[53] and (2) a study reported reduced levels of HA in the airway smooth muscle cells from patients with asthma and COPD. This study reaffirms the prior evidence of depletion of HA in lungs of patients with COPD.[54]

A recent study in 11 patients with COPD was carried out to determine safety and for measurement of a change in elastin degradation by measuring DI levels. Eight patients received HA 0.01% aerosol morning and night for 14 days, and three patients received placebo. No significant adverse events were reported.[55]

Most significantly, levels of the biomarker DI in this study were significantly reduced over their baseline levels in the patients who received medication. This result could be predicted from our earlier animal and in vitro studies in which we observed the effect of HA reducing the elastase degradation of elastin. This study also demonstrated that the biomarker can be used as an index of a biological effect of this therapy on lung elastin.

At present there is a clinical trial under way (ClinicalTrials.gov: NCT03114020) in 40 patients with AATD and pulmonary emphysema not receiving augmentation therapy. Twenty patients will receive 0.03% HA aerosol twice a day for 28 days, and 20 patients will receive placebo. The end points are tolerance to the medication, stability of lung function, and reductions in DI biomarkers in plasma, urine, and sputum. Subsequent trials will hopefully be conducted for longer periods of administration and include CT evidence of preservation of lung structure. Also, consideration should be given to including patients with non-AATD-related COPD. HA aerosol as a therapy in COPD would be initiated at the earliest diagnostic evidence of COPD in the hope of limiting its progression.

Biological Effects of Small Fragments of HA

Several studies over the past 20 years have indicated that small fragments of HA contribute to the immune cell response in inflammatory sites through receptors CD44, RHAMM (receptor for hyaluronan-mediated motility), and Toll-like receptor 4.[56,57] Low-molecular-weight fragments have been shown to stimulate mouse alveolar macrophages to produce RNAs of several chemokines and cytokines including metalloelastase. Opposite effects were induced by high-molecular-weight fragments that suppressed such expression.[58-61]

The differing results of studies using high-molecular-weight HA as compared with lower molecular weights have no straightforward explanation. Camenisch and McDonald suggest that the varying response may depend on the reaction of HA of varying size with its receptor.[62] A factor to be considered is the possible contamination by DNA in preparations of HA produced from sources such as rooster comb, umbilical cord, and gram-positive streptococci, which can have a nanogram range of contaminants that produce effects that are not HA induced. Filion and Phillips[63] demonstrated that in preparations of HA that induced synthesis of interleukin 12 and tumor necrosis factor, treatment of such preparations with DNAse abrogated the synthesis of these substances. It is also noteworthy that exposure of mice to HA aerosols with a mean molecular weight of 150,000 Da for several months has not induced any respiratory inflammatory reactions.[40]

With respect to the safety of HA aerosol preparations, there is a study of the use of 7% hypertonic saline containing 0.1% HA administered to patients with cystic fibrosis twice per day for 28 days. Patients found the 7% hypertonic saline solution more tolerable when it contained HA. No adverse results on lung function or clinical state were reported.[64] A systematic review of the literature on the use of topical HA preparations in the pediatric population with upper airway inflammatory disease and cystic fibrosis found no clinical adverse effects and positive clinical results.[65]

A study of the effect of hypertonic saline aerosol containing 0.1% HA molecular weight 0.3 to 0.5 $\times 10^6$ Da in patients with cystic fibrosis for 4 to 6 weeks showed no increase in sputum cytokines and concluded that the HA did not adversely affect the status of airways in cystic fibrosis.[66]

It should be noted that non-AATD COPD is a complex disease with varying clinical phenotypes; however, biomarker studies indicate increased lung elastin degradation, and anatomic studies of lungs with COPD show significant structural abnormalities of elastin throughout the lung. Despite phenotypic variations in non-AATD COPD, it remains possible that anatomically, elastin degradation remains a significant component of pathogenesis and might be amenable to agents that can limit such degradation and preserve lung structure.

Conclusions

Long-term studies in AATD of the effects of augmentation therapy on lung tissue density and lung elastin degradation demonstrate a slowing of the development of pulmonary emphysema. Studies of HA aerosol in animal models and in preliminary human studies indicate a protective effect of HA, which possibly functions as a barrier to prevent elastase degradation of elastin in situ. This aerosol, which is replacing a deficient glycosaminoglycan in the lung parenchyma in patients with COPD, deserves consideration for further development as a therapy in COPD related to AATD and COPD not related to AATD.

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