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Original article

Aerosolized hyaluronic acid and its applications

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Abstract. Although the interaction of low MW HA fragments with surface receptors such as TLRs may explain how HA can favor the inflammatory response, some physical and chemical properties, such as high MW HA, may increase its capability to retain water and to preserve the distensibility of elastic fibers that is dependent on the interaction with water molecules, thus enhancing pulmonary ventilation. Encouraging clinical results provide the beneficial effect of inhaled HA in CF patients who have previously shown poor tolerance to aerosolized hypertonic saline solution. (www.actabiomedica.it)

Key words: hyaluronic acid, respiratory disease, inhaled therapy

Introduction

Cystic Fibrosis (CF), the most common lethal genetic disorder in the Caucasian population, is caused by mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes for a cAMP-dependent chloride channel expressed in secretory epithelia of the body. High morbidity and mortality are due to chronic obstructive lung disease resulting from airway surface dehydration and impaired mucociliary clearance that lead to airway mucus obstruction, neutrophilic inflammation and bacterial infection. As a consequence, early onset of bronchiectasis and progressive emphysema are a characteristic feature of CF lung disease and their severity contributes to progressive loss of lung function and disease burden in patients with CF. While the imbalance between chloride secretion and sodium absorption in submucosal glands and airway epithelium in the lung is responsible for detrimental airway dehydration, cells lacking functional CFTR display several other constitutive dysfunctions such as unregulated activation of the nuclear factor kappa-light chain-enhancer of activated B cells (NF-κB) pathway (hyper-inflammation)

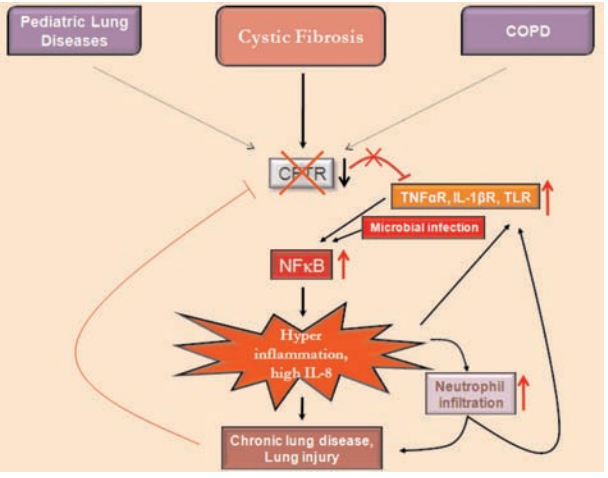

Figure 1. Pathogenetic cascade derived from decrease of CFTR function and enhanced NFkB activation from Discov Med 2010

(Fig 1) (1), decreased anti-inflammatory responses, and oxidative stress that is a hallmark in CF lung disease and represents an inducer of the pro-inflammatory status, upon stimulation with either Toll-like receptor (TLR) ligands or Pseudomonas aeruginosa. Bacterial products such as lipopolysaccharide (LPS), which trigger the recruitment of macrophages and

neutrophils, and increase secretion of elastolytic proteases, such as macrophage elastase and neutrophil elastase, may also play an important role in emphysema formation in CF (2). Elevated levels of interleukin-8 (IL-8) signaling mediated by the NF-κB result in chronic infection, neutrophilic inflammation, and progressive structural lung damage. This pathogenetic cascade indicates that additional rehydration strategies may be required for effective treatment of airway mucus obstruction in CF with secondary consequences also on inflammation (1).

Discussion

Glycosaminoglycans (GAGs) play key structural roles in the lung where they are distributed in the extracellular matrix that fills the interstitial space lying between the capillary endothelium and the alveolar epithelium (Fig. 2) (3), in the subepithelial tissue, bronchial walls, and airway secretions. GAGs are also involved in a number of biological activities mediated by specific receptors including cell migration and differentiation, antigen recognition, cell adhesion and communication. Recently (3), the importance of glycosaminoglycans (GAGs) in CF has been highlighted. Increased concentrations of GAGs have been found in BALF from children with CF, and secretion of HA is markedly increased in bronchial cells and CF tissues (4).

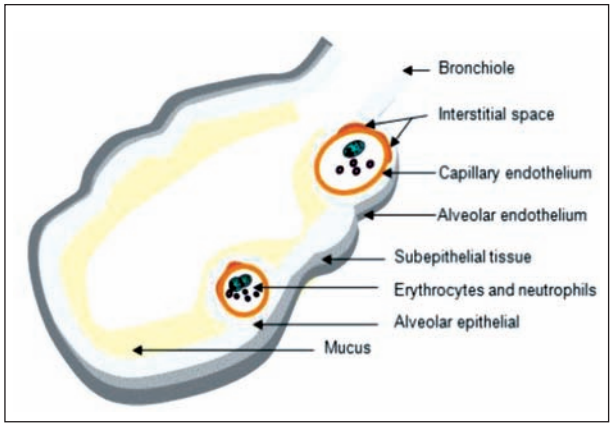

Figure 2. Localization of extracellular matrix in the lung from Scientific World Journal 2011

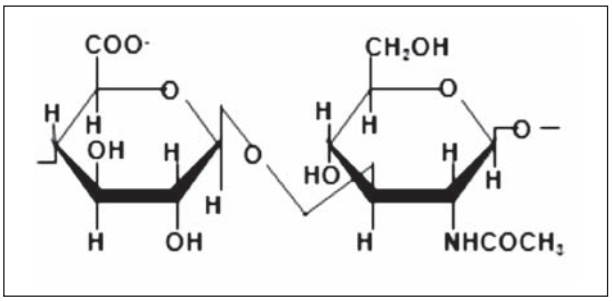

Figure 3. Structural formula of HA

HA is a simple linear polysaccharide chain belonging to the family of glycosaminoglycans made of alternating β-1, 4-glucuronic acid and β-1, 3-N-acetylglucosamine disaccharide units (Fig. 3). It occurs in many tissues and body fluids in vertebrates. Previous experimental results in animal models have provided effects of aerosolized HA in preventing experimentally induced parenchymal emphysema that involves elastic fiber injury (5). The intratracheal administration of hyaluronan prior to the induction of experimental emphysema developed significantly less disease than untreated controls.

The use of aerosolized hyaluronan (HA) has been proposed as an alternative approach to treating emphysema (6).The protective effect of HA may be related to its ability to bind to lung elastic fibers, thereby preventing their breakdown by elastases (7). In particular, it has been proposed that the special ability of this polysaccharide to retain water may increase the elasticity of lung elastic fibers, producing a relatively rapid improvement in pulmonary mechanics. However, the biological activity of HA is likely related to its molecular size, with larger polysaccharide chains having anti-inflammatory properties and smaller ones having pro-inflammatory activity.

No nebulized formulations including HA have been provided in patients with chronic obstruction pulmonary disease in recent clinical studies. Such a potential beneficial treatment of nebulized HA for respiratory diseases in clinical trials is not expected to become evident as a measurable effect for at least several years, taking into account that pulmonary emphysema progresses at a relatively slow rate.

In CF, early onset and progressive emphysema is a characteristic feature of lung disease, as recently

shown by non-invasive methods (8), where a pivotal role in the destruction of lung tissue is played by release of proteolytic enzymes that overwhelm the antiprotease defences of the lung. While cytokine- and chemokine-GAGs interactions seem to decrease the plasma clearance and increase the cytokine activity (9) where several fragmentary ECM components are pro-inflammatory such as low molecular weight HA (LMWHA), intact high molecular weight HA (HMWHA) can inhibit TLR-2 signaling *in vitro* and *in vivo* (10) playing an active role in the maintenance of immune tolerance (10).

Clinical application of HMWHA has been recently proposed (11) for increasing tolerability of inhaled hypertonic saline solution mucolytic agent. Several studies (12, 13) have further confirmed this data in controlled clinical trials. In addition, both mouse models CftrF508del mice, carrying the most common F508del-CFTR mutation, and Scnn1b-Tg mice, overexpressing the **b**-subunit of the ENaC channel, were analyzed to evaluate the expression of markers of inflammation in lung homogenates after nebulized HA. In particular, decreased levels of TNFa and MIP-2, the analogous of human IL-8, mainly responsible for neutrophil recruitment into lungs, reducing leukocyte infiltration in the lungs of mice, and decreasing levels of MPO, an enzyme that is most abundantly present in neutrophils, were found (15). The effect of nebulized HA in controlling ROS-mediated effects in CF epithelia induced by the challenge with PA-LPS might help preventing HA degradation, thus avoiding the putative pro-inflammatory effects of small HA fragments.

Conclusion

Aerosol HA administration may have a potential therapeutic impact on respiratory diseases reducing the bronchial hyper-reactivity to muscular exercise in asthmatics and controlling progression of emphysema. Preliminary results in mouse models could pave the way for the implementation of nebulized HA not only as adjuvant in medical devices, but also as a putatively safe, anti-inflammatory medical device for the treatment of patients with CF.

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