



Hyaluronidase: A Potential New Treatment for Acute Respiratory Distress Syndrome

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Abstract

Acute respiratory distress syndrome, also known as diffuse alveolar damage, is an acute injury to the lungs. Patients experience severe shortness of breath and require mechanical ventilation. It is not a specific disease, but an acute lung dysfunction associated with a variety of disorders: pneumonia, shock, sepsis, and trauma. A similar lesion occurs in newborn infants, called hyaline disease of the newborn. It occurs in premature babies and has the same pathophysiological mechanism as acute respiratory distress syndrome. Hyaline membranes are a pathologic feature of acute respiratory distress syndrome, consisting of basophilic structures that coat alveolar surfaces. They prevent oxygen exchange and are the basis of the lethality of this disorder. The syndrome is associated with very high levels of hyaluronan in broncho-alveolar lavage specimens. We postulate that the hyaline membranes of acute respiratory distress syndrome are hyaluronan-rich structures associated with serum hyaluronan-binding proteins such as fibrinogen and fibrin. Potent infectious influenza viruses are recurrent pandemics and potential terrorist threats. Lethality of influenza infection correlates with the presence of hyaline membranes. Installation of hyaluronidase as an aerosol would provide a new treatment for acute respiratory distress syndrome, for which there has been no new treatment in 45 years. The pig is the only species other than humans that develop hyaline membranes. Employing this treatment in the porcine model would provide a direct test of the hypothesis.

Keywords: Hyaluronidase; Acute respiratory distress syndrome

Abbreviations: ARDS: Acute Respiratory Distress Syndrome; BAL: Broncho-Alveolar Lavage; BSL: Biosafety Laboratory; ECM: Extracellular Matrix; GAG: Glycosaminoglycan; HA: Hyaluronan, Hyaluronic acid; H&E: Hematoxylin and Eosin; 4-MB: 4-methylumbelliferone; NRDS: Neonatal Respiratory Distress Syndrome; PEG: Polyethylene-Glycol; SARS: Severe Acute Respiratory Syndrome

Introduction

Acute respiratory distress syndrome (ARDS) is a clinical phenomenon [1] first described in 1967 [2]. A set of recalcitrant respiratory disorders had been observed in a variety of patients that did not respond to conventional therapies. ARDS is often a lethal disease. No new treatment modality has been devised, even though this is a wide spread problem and despite many efforts. A therapy for ARDS is proposed here based on an aerosol of a recombinant enzyme. The logistics behind such an approach is presented.

ARDS and hyaline membranes

ARDS is not considered a specific disease, but a disorder in which there is acute lung dysfunction. It is associated with a variety of disorders including pneumonia, shock, sepsis, chemical injury, and trauma. It is one of the most severe forms of acute lung injury. Current figures estimate that 200,000 cases occur per year in the US, with a mortality rate of approximately 40% [3].

Since it is not a single disorder, a task force of pulmonary experts in 2011 developed the "Berlin Definition" [4]. They proposed three categories of ARDS based on degree of hypoxemia: mild, moderate and severe. These were associated with increasing mortality, and thus, will provide better informed clinical care and a more empirical patient evaluation.

One of the characteristic features of ARDS in the human is the development of hyaline membranes. This is a histologic feature of pulmonary tissue from ARDS patients. Using haematoxylin and eosin (H&E) staining, it is an amorphous pink acellular ribbon-like material that binds to the walls of alveoli. These structures are thought to prevent

air exchange between alveoli and capillaries imbedded in alveolar walls. The clinical and pathological features closely resemble those observed in premature infants, referred to as neonatal respiratory distress syndrome (NRDS). Similar structures occur in the lungs of preterm NRDS infants.

Neonatal respiratory distress syndrome

NRDS, formerly known as hyaline membrane disease of the newborn, is a serious respiratory disorder in the preterm infant [5]. The syndrome is attributed to a deficiency in surfactant production and structural immaturity of the lungs. It is the most common cause of death in these preterm infants. An animal model has been obtained in premature monkeys [6,7] that closely resembles the human disorder. It is assumed that a similar pathophysiological mechanism is involved.

Influenza, past influenza epidemics and pandemics

Influenza is a negative stranded RNA virus. There have been many virulent pandemics of influenza in the past. The most notorious is the Spanish flu of 1918-1919. More people died of Spanish influenza in a single year than in four-years of WWI, with a mortality rate of about 40%. Approximately 3% of the world's population died, making it one of the deadliest natural disasters in human history, with more deaths than in the Black Death-Bubonic Plague of 1347 to 1351. More recent epidemics include severe acute respiratory syndrome (SARS) of 2002-2003, and H1N1, known as the Swine flu of 2009, with 18,000 deaths worldwide.

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Terrorist threats and anti-terrorist measures

Genetically engineered influenza viruses have the potential of becoming a basis of biological warfare and a major terrorist threat. Preparation of such viruses for use in biological warfare is relatively straight-forward. A high school biology class could accomplish this bit of molecular genetics. Thus, it is absolutely necessary to become prepared for such events, and to evolve protective strategies for such contingencies. The present hypothesis provides precisely such a measure. The hyaluronidase aerosol would be a universally effective treatment modality, instantly available for all victims of such a potential catastrophe.

Animal models for influenza infections and ARDS

A number of animal models are available for studying influenza infections as well as for ARDS. The ferret reflects closely most features of the human disease. They are exquisitely susceptible to infection with human influenza viruses, express similar symptoms, and are the ideal small animal model for research [8,9]. They also respond in a fashion similar to humans the various immune responses including vaccination strategies. Other animal models include rats, hamsters, and guinea pigs.

However none of these animals develop the hyaline membranes that characterize ARDS in humans. The mortality of influenza infections in humans correlates with hyaline membrane formation. Those that succumb to infection have hyaline membranes, those that survive do not on a nearly one to one basis (personal observations, RS). There is only one experimental animal that does develop hyaline membranes, and that is the pig [10]. No other influenza non-primate model does so. Hyaline membranes in the pig have pathologic and histochemical features identical to that in the human. There is one exception, and that is the animal model for NRDS, the premature monkey [7].

The Mendelson syndrome for ARDS and hyaline membrane formation

A major problem for the use of pigs in studying influenza-derived ARDS and hyaline membranes is human infectivity. Pigs and humans are susceptible to the same viruses. Influenza viruses exchange antigenic epitopes easily and rapidly. Virulent infections may arise, even when attenuated viruses are utilized initially. For this reason, a Biosafety Laboratory-3 (BSL-3) facility is required. This is extremely expensive, and very cumbersome for animal manipulation. Even small pig models such as Yucatan pigs, micro-pigs, or young piglets present difficulties. For this reason, a non-infective ARDS model is preferable.

The Mendelson syndrome, the aspiration of gastric contents into the lungs elicits hyaline membrane formation [11]. Inoculation of 0.1 M HCl into the pig through a bronchial tube reproduces the syndrome rapidly and easily [10]. This provides a convenient non-infectious alternative to influenza infection for studying hyaline membranes.

Hyaluronan

Hyaluronan (HA, hyaluronic acid) is a straight chain glycosaminoglycan (GAG) of the extracellular matrix (ECM) [12-14]. It is the major component of the ECM, particularly prominent during development, regeneration and repair, and whenever there is rapid tissue turnover. HA characterizes the stem cell niche [15]. HA is the only GAG that has no secondary modifications. It is not sulfated, nor does it undergo epimerization reactions. HA also characterizes tissues with infections and other inflammatory responses [16,17]. Evidence suggests that hyaline membranes are HA-rich structures.

Hyaluronan in ARDS

There are major concentrations of HA that occur in ARDS [2]. Samples taken from broncho-alveolar lavage (BAL) specimens of ARDS patients have markedly elevated HA levels. Less than 20 µg/ml of HA occur in BAL samples from patients with normal lungs, while in two studies, levels of 353 µg/ml and 515 µg/ml were observed, representing 20 to 25 times normal levels [18,19]. Increased HA deposition also occurs in the premature monkey model of ARDS [7]. An excellent review of the role of HA in acute lung injury is available [20]. Such data suggests that hyaline membranes contain HA. There are copious levels of HA in the inflamed lung, together with several HA-binding proteins. Such proteins are present in plasma and in tissue exudates, which are filtrates of plasma. Prominent among such HA-binding proteins are fibrinogen, fibrin and its split products [21-23], and inter-alpha-inhibitor protein family [24]. The latter not only stabilizes ECM structures such as hyaline membranes, but is also hyaluronidase inhibitors [25]. All of these features contribute to the argument that hyaline membranes are stabilized HA-rich structures that are protected from the surrounding catabolic activities that characterize inflammation.

Suppression of HA synthesis ameliorates acute lung injuries

Further evidence for the role of HA in acute lung injury comes from inhibitor studies. The drug 4-methylumbelliferone (4-MB) is a potent inhibitor of hyaluronan synthesis [26]. Administered orally in mice, it suppresses the intensity of the inflammatory response following acute lung injury [27,28]. This provides additional evidence that HA is associated with the inflammation in acute lung injury. The 4-MB is available as a therapeutic in Europe, but not yet in the U.S.

Hyaline

The term "hyaline" used by Pathologists, has no biochemical equivalent. It was described 150 years ago by German pathologists before there was anything such thing as biochemistry. It was observed by H&E staining, and various special stains, all fall-outs from the aniline dye industry. A hyaline substance appears glassy and pink after staining and is acellular. There have been no attempts to analyse hyaline biochemically.

Hyaline therefore is an ambiguous term. We postulate that hyaline staining may refer to HA-rich structures. This can be confirmed by preincubation of tissue sections with a hyaluronidase. The proviso is that most hyaluronidases are also chondroitinases that also degrade chondroitin sulphate, albeit at a much slower rate. There is one bacterial enzyme that is HA-specific, the one derived from *Streptomyces hyaluronolyticus*. Many histochemical studies have failed to use this enzyme. A step towards evaluating the HA content of hyaline membranes in Pathology specimens would be to demonstrate loss of staining of tissue sections following *S. hyaluronolyticus* incubation.

Hyaluronidases

Hyaluronidases are the hydrolase enzymes that catabolize HA throughout the body of vertebrate. They are hydrolases rather than the eliminases that occur in prokaryotes. The intrinsic difficulty in measuring such hydrolases was the reason these were long neglected enzymes [29]. This is in marked contrast with the bacterial enzyme activity that can easily be followed by spectrophotometry. There are six hyaluronidase-like sequences in the human genome, tightly clustered, three each on chromosomes 3p and 7q [30,31]. Of these, two are acid-active and are prominent in HA degradation in somatic tissues,

HYAL1 and HYAL2. Both of these are present in the 3p cluster. The third hyaluronidase-like sequence in this cluster is not associated with a detectable enzyme activity. In the 7q gene cluster, one is a pseudogene, transcribed but not translated. Another is a chondroitinase [32,33]. pH-20 appears to be the only hyaluronidase enzyme active at neutral pH. It is associated with sperm and is involved in fertilization. The latter enzyme has been commercialized in a recombinant form, and is available as Hylenex[®] [34,35], a product of Halozyme Therapeutics, San Diego, CA. A polyethylene-glycol cross-linked (PEGylated) form of the enzyme is also being produced. The latter formulation has considerably greater half-life following its administration.

Aerosols as drug treatment modalities

Hyaluronidase as a therapeutic agent administered as a nasal spray for pulmonary delivery has not been attempted previously. The pulmonary administration of medications through nasal aerosols has been in use for decades. Corticosteroid inhalants are employed to alleviate asthma and for patients with chronic obstructive pulmonary disease [36]. There are many other successful examples of such uses. Nano particles have been used for pulmonary drug delivery [37,38], as well as spray-dried particles [39]. Enzymes have been utilized previously as aerosol treatments. One of these is Pulmozyme[®], a cocktail of human recombinant deoxyribonuclease (DNAases or rhDNase) in the management of cystic fibrosis. A precedent exists therefore for the use of a hyaluronidase such as Hylenex[®] as a mode of treatment. This has the potential of becoming a new and effective treatment for ARDS.

Serious provisos and potential drawbacks

The lung is an HA-rich structure, and even more so when there is inflammation [40,16]. A serious drawback to our hypothesis, that hyaluronidase can be a therapeutic measure for the treatment of ARDS, is that it may make the condition even worse. Administered enzyme could fragment existing HA-rich structures of the lung, and thus intensify disease. Endogenous HA and its HA fragment degradation products would flood the lung. Small HA polymers are highly angiogenic, inflammatory, and immune-stimulatory [41,42]. HA fragments are themselves not only intrinsically inflammatory, but also induce inflammatory cytokine expression [43,44]. It is conceivable that the hyaluronidase enzyme treatment may intensify rather than abate the inflammatory reaction. Thus there may be serious consequences of hyaluronidase administration. This is a situation that would be readily apparent in the porcine model using the Mendelson syndrome. Titration of the volume of the HCl solution administered in the pig model may be able to reproduce the three levels of severity of ARDS: mild, moderate and severe. The levels of hyaluronidase aerosol provided could perhaps be modulated to maximize clinical response and also to avoid potential adverse reactions.

Conclusion

It is postulated that the hyaline membranes in patients with ARDS are HA-rich structures. This is an insoluble complex of HA together with plasma HA-binding proteins that adhere to inflamed alveoli preventing oxygen exchange. A recombinant form of the human hyaluronidase enzyme PH20 can be administered as an aerosol to ARDS patients. This will degrade hyaline membranes in these damaged lungs and increase survival of such patients. It may become the first effective treatment modality in several decades for ARDS in adults and may also be effective for NRDS in premature infants. A porcine model for ARDS, employing bronchial delivery of 0.1 M HCl to the lungs, makes it possible to test this hypothesis.

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