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PERSPECTIVE

Novel Therapeutic Uses of Alpha-1 Antitrypsin: A Window to the Future

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Abstract

Alpha-1 antitrypsin, a potent serine protease inhibitor, has been used as augmentation therapy in patients with alpha-1 antitrypsin deficiency for many years. Recent research into the diverse anti-inflammatory, immune-modulatory and tissue-protective actions of alpha-1 antitrypsin has raised the possibility of broadening the therapeutic spectrum of alpha-1 antitrypsin to include diseases other than alpha-1 antitrypsin deficiency. The purpose of the workshop was to summarize the results of basic investigations and, if available, clinical studies in which the effects of alpha-1 antitrypsin were explored in relation to clinical conditions that are not associated with alpha-1 antitrypsin deficiency. Included among these are type 1 diabetes, cell/organ rejection, viral infection, cystic fibrosis, bronchiectasis/COPD, heart failure, Crohn's disease and connective tissue diseases. Although the therapeutic utility of alpha-1 antitrypsin in these conditions remains to be established, the existing data suggest that this protein eventually will become a treatment option in several diseases some of which are not rare. At present, only human plasma-derived alpha-1 antitrypsin is available for clinical use. Given the limited supply and the potential for extended use of this product, there will be a need for new formulations of alpha-1 antitrypsin in the future. Therefore, the prospect of finding new sources and airway delivery methods of alpha-1 antitrypsin were also discussed. The presentations at the meeting addressed the scientific basis for new clinical applications of alpha-1 antitrypsin and the regulatory requirements needed to bring this therapeutic protein to a wider range of patient populations.

Keywords: alpha-1 antitrypsin, cystic fibrosis, COPD, organ rejection, viral infection, type I diabetes, crohn's disease, inflammation

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Introduction

Alpha-1 antitrypsin (AAT), a potent serine protease inhibitor, has been used as augmentation therapy in patients with alpha-1 antitrypsin deficiency for many years. Recent research into the diverse actions of AAT has raised the possibility of broadening its therapeutic spectrum to include other diseases. Researchers convened at the Alpha-1 Foundation's 12th Gordon L Snider Critical Issues Workshop to summarize the results of basic investigations and, if available, clinical studies in which the effects of AAT were explored in relation to clinical conditions that are not associated with AAT deficiency. Included among these were diabetes, cell/organ rejection, cardiovascular disease, viral infection, cystic fibrosis, bronchiectasis/COPD, Crohn's disease and connective tissue diseases. Although many observations are still at the basic science level, several proof-of-concept studies have already been completed or initiated.

The workshop had three aims. The first was to summarize what is currently known about potentially beneficial effects of AAT in animal models of a variety of human conditions that are not associated with AAT deficiency, and to highlight early clinical studies involving several diseases not caused by AATD deficiency. The second aim was to discuss the potential for developing recombinant or transgenic AAT as substitutes for human plasma-derived product, and for formulating AAT as an aerosol for the treatment of inflammatory lung diseases. The third aim was to discuss regulatory issues pertaining to new therapeutic indications for AAT and the introduction of new AAT formulations for clinical use.

The following summary of the workshop proceedings is divided into three topics: New applications of AAT, new AAT formulations, and regulatory considerations. Publications relevant to the discussions at the meeting are not referenced in the text. Instead, the summary is followed by a list of thematically grouped references submitted by the speakers.

New Applications of AAT

Basic investigations

Type-1 diabetes and other immune-mediated diseases

Type-1 diabetes mellitus (DM) is an autoimmune condition characterized by an inflammation-driven attack on beta cells in the pancreas, resulting in fluctuating blood sugar levels. The pancreas responds to injury by releasing potent inflammatory products that, through a positive feedback cycle of inflammation, continue to cause damage to pancreatic cells. AAT is an endogenous anti-inflammatory and tissue-protective molecule that could potentially interfere with this inflammatory process, but studies have shown that endogenous circulating AAT is less active in diabetic individuals, potentially due to high glucose levels in hepatocytes where AAT is therefore excessively glycated.

A number of studies have investigated how AAT can suppress the pancreatic inflammation associated with DM. AAT gene therapy prevented the development of hyperglycemia in non-obese diabetic mice, and when clinical-grade AAT was added to pancreatic islets, it reduced inflammation by protecting islets against inflammatory cytokines, fatty acid toxicity, and high-mobility group protein B1 (HMGB1)-induced injury. AAT also blocked the release of inflammatory molecules such as nitric oxide, chemokines and pro-inflammatory cytokines.

Furthermore, AAT inhibited caspase-3, protected beta cells from apoptosis, and increased insulin production in diabetic mice. When AAT was injected every 3 days at 60 mg/kg to diabetic mice, blood glucose levels dropped and remained low even after treatment stopped. These findings form the basis of currently running human AAT treatment trials in type-1 diabetes. AAT also has been shown to modify other immune-mediated responses including cell and organ rejection through various mechanisms.

In the presence of AAT, macrophage co-stimulatory molecule expression is reduced, and the release of IL-1 receptor antagonist (IL-1Ra) is increased, resulting in decreased islet injury and free fatty acid-related inflammation. AAT can inhibit B lymphocyte isotype-switching in vivo and diminish antigen-induced increases in serum IgG levels. Possibly as a result of these actions, AAT has been shown to protect islet allografts from acute rejection in immune-intact animals. It was also demonstrated that AAT facilitates graft survival by promoting angiogenesis through an increase in CD31 and vascular endothelial growth factor (VEGF). Although in their early stages, these studies are encouraging and the use of AAT to suppress rejection of transplanted cells or organs deserves further exploration.

It has been reported that AAT can decrease autoantibody levels and delay the development of arthritis in collagen-induced arthritic mouse models. Other immune-mediated disease models in animals in which AAT was used include Crohn's disease, graft vs. host disease (GVHD) and acute myocardial infarction. The SAMP1/Yit mouse is used as a model for human Crohn's disease because this mouse develops a spontaneous ileitis analogous to that observed in humans. Treatment of SAMP1/Yit mice with a human plasma-derived AAT reversed intestinal lesions, improved epithelial barrier function, and attenuated cytokine production in colon tissue explants.

In the GVHD mouse model, AAT improved survival rates in animals with mismatched bone marrows. Finally, in a mouse model of acute myocardial infarction, loss of ventricular function was prevented when AAT was administered after the myocardial infarction had occurred. The therapeutic potential of AAT in humans with these conditions remain to be investigated.

Viral diseases

Viruses can be highly adaptive and elude the immune system. Because viruses neither metabolize nor respond actively to external conditions, host molecules are appropriated to enable infection and replication. This is illustrated for Influenza virus (Flu), which requires function of host serine proteases to infect cells. The Flu spike protein hemagglutinin facilitates binding to host cells only after activation by host serine proteases. Specific serine proteases that are restricted to lung tissues process an external hemagglutinin fragment and enable the binding of hemagglutinin to host cell receptors. This activation event may, in part, explain the tropism of Flu virus to infect lung tissues. Because AAT is a potent serine protease inhibitor, it can block activation of Flu hemagglutinin and prevent binding to cells, and viral replication.

In an *in vitro* model of H1N1 Flu infection, exogenous AAT suppressed viral infection by 99%. Studies in a mouse model of Flu pneumonia showed that mice over-expressing human AAT in lung tissues were resistant to weight loss and mortality compared to wild-type

control animals. In an epidemiological study of human Flu infection comparing persons without or with AAT deficiency, AAT deficiency was a significant risk factor for infection. These observations suggest that AAT may have use as a treatment or prophylactic agent for Flu. Since immutable host-derived molecules (lung serine proteases) are targeted, AAT-based therapy may be impervious to viral mutation-induced resistance.

AAT has also been evaluated as a possible endogenous human immunodeficiency virus type 1 (HIV) antagonist. HIV does not replicate in healthy whole blood, suggesting the presence of natural antiretroviral substances. AAT may be an endogenous antiretroviral molecule since AAT potently suppressed (up to 100%) HIV production in chronically infected monocytic cells, inhibited HIV infection in a cell line designed to detect HIV cell entry, and suppressed virus replication in primary human peripheral blood mononuclear cells (PBMC) infected with HIV.

Although HIV did not replicate in blood obtained from healthy volunteers, HIV replicated prodigiously in blood from AAT-deficient persons. Because AAT suppresses Flu and HIV replication in several studies that used AAT at physiological concentrations, AAT may function naturally as an innate immune inhibitor of a broad range of viral infections. Could AAT be used to treat Flu or HIV infection? This question thus far has not been addressed in clinical trials but may deserve further consideration.

Non-alpha-1 lung disease

Cystic fibrosis (CF) is characterized by epithelial ion channel abnormalities that lead to impairment of host defenses, bacterial colonization and lung disease, which is the leading cause of mortality associated with CF. In this condition, lung neutrophils are abundant, intrinsically abnormal and more potent than neutrophils obtained from healthy individuals or patients with non-CF bronchiectasis. Neutrophil elastase (NE) is a potent serine protease secreted by neutrophils that severely impacts the airway. It causes direct epithelial damage, increases mucous secretion and decreases ciliary clearance and other host defenses.

NE also upregulates IL-8 and neutrophil chemoattractants, leading to a cycle of increasing inflammation. Its proteolytic effects act on complement receptors and immunoglobulins, and it can upregulate/activate other proteases. Increased NE release is a risk factor for a virulent pseudomonas aeruginosa lung infection due to it rendering neutrophils less efficient in killing the bacterium. AAT can inhibit NE and lead to restoration of the normal host defense by preventing the degradation of defense receptors and by down-regulating other proteases that impair host defenses.

CF patients have been shown to have normal AAT levels in serum and in the lung, but in the lung AAT is inactivated by proteolytic cleavage, binding to proteases and by oxidation of the molecule's active site methionines. This has led to clues as to the cause of other lung diseases and the potential for therapy with AAT. In non-AATD emphysema AAT can be inactivated by oxidants, in cigarette smoke, thereby diminishing its anti-NE activity. It has been shown that the concentration of methionine sulfoxide is increased in the bronchoalveolar lavage fluid of smokers and that this was associated with a reduced AAT activity against NE. There currently is no definitive treatment for CF or non AATD emphysema but the anti-protease properties of AAT demonstrate that it shows promise as a treatment option, particularly for CF at this point in time.

Clinical investigations

Human plasma-derived AAT has been in clinical use since 1988, and has proven to be safe and well-tolerated when administered intravenously. Several such products are FDA approved, but only for patients with AAT deficiency. As alternate sources of AAT are not currently available, early clinical studies in conditions other than AAT deficiency have made use of human plasma-derived AAT.

Aerosol AAT for Non-Alpha-1 Lung Disease

The current treatment paradigm for CF focuses on alleviating symptoms and preventing infections. AAT has been shown to have antiprotease, anti-inflammatory, immunomodulatory and antimicrobial properties, making it a strong therapeutic candidate. In vitro CF sputum shows reduced NE activity when treated with exogenous AAT. Drug companies therefore are researching aerosolized AAT formulations as a possible new treatment option for AAT deficiency-related lung disease and other inflammatory lung conditions such as CF.

Particle size, deposition rate, delivery system and formulation stability are key factors in the effectiveness of an aerosolized drug. Studies have shown that particle diameter must be between 1 and 5 μm for optimum deposition. Nebulizers are required for aerosol AAT therapy, and highly efficient nebulizers including breath-activated models have been developed that improve intrathoracic drug deposition independent of lung function, delivering more than 70% of the filling dose to the lungs. Furthermore, studies demonstrated that AAT was found in the lungs of patients in an active form and it was capable of crossing the alveolar-capillary membrane and diffuse into the blood. AAT has proven to be biochemically active after aerosolization with several devices.

Studies with aerosolized human AAT in CF patients have provided evidence for complete inhibition of NE activity in epithelial lining fluid. Also, a high-purity liquid formulation of AAT has been developed for aerosol treatment. Five separate clinical studies have been completed, and a Phase 2 study has explored the safety and efficacy of inhaled AAT in CF patients. Another Phase 2 repeated dose study showed a decrease in sputum neutrophil counts, unopposed elastase activity and IL-8 concentration. It was concluded that the aerosol AAT

formulation was safe, well tolerated for up to 28 days, and reduced inflammatory markers in CF patients.

Type-1 diabetes

Three trials are currently running in which AAT is administered to recently diagnosed type 1 diabetes patients: NIH clinical trial registry codes NCT01304537, NCT01319331 and NCT01183468. The outcomes were pending at the time of the workshop.

New AAT Formulations

Although inhaled AAT is considered a new formulation and, if clinically effective, would reduce the amount of protein needed to treat lung diseases other than AAT deficiency, alternate sources of AAT may be needed to meet the increasing demand for this protein. Furthermore, new indications for systemically administered AAT will further increase the demand for additional product. Several companies have used transgenic or recombinant approaches to produce AAT. A major challenge in these investigations has been the requirement to mimic the glycosylation pattern of the native human AAT to ensure adequate tissue penetration and acceptable plasma half-life values. Recently, a recombinant AAT formulation has been developed that has achieved near identical glycosylation when compared to plasma-derived AAT. However, further studies are warranted to demonstrate the clinical safety and efficacy of this and similar products.

Regulatory Considerations

Both human plasma-derived aerosol AAT and recombinant or transgenic formulations will have to undergo regulatory review. If licensed, AAT could be the first large protein delivered by inhalation. New dose formulations have the potential to change adverse effects profiles and the risk to benefit calculation, potentially affecting drug approval. In vitro and in vivo data sets that support appropriate safety and immunogenicity parameters are imperative if the aerosol AAT is to become FDA approved. Presently, the only approved AAT formulation is intravenous. The November 2005 Blood Products Advisory Committee voted to require new AAT products for AAT deficiency to provide clinically meaningful endpoints, such as pulmonary function or serial lung density changes by CT, exacerbations of COPD, and mortality, not just surrogate endpoints such as AAT levels.

This applies to intravenous and aerosol AAT in AAT deficiency and presumably in other diseases as well. Of note, while the most striking attribute of AAT is anti-inflammation, it should not be regarded as such regulatory-wise, as it stands alone in the ability to modulate the immune system instead of suppressing it, and also protecting tissues from enzymatic degradation; thus, it cannot be narrowed-down to one function for the purpose of regulatory definitions.

A number of new AAT sources are being explored, and it is important for researchers to provide comprehensive data if the new drugs are to make it to clinical trials and ultimately to the market. Significant biomarkers must be established with corresponding validated assays to determine important biological parameters such as immunogenicity, adverse events and efficacy. Additionally, studies determining appropriate dosing guidelines, pharmacokinetics and pharmacodynamics should be completed to further support new AAT formulations in the approval process. These requirements, while needed, will have a major role in bringing new AAT products to the market and licensing them for new clinical applications.

Conclusions

An unbiased assessment of the data presented at the workshop leads to several conclusions about the prospect of administering AAT therapeutically to patients suffering from conditions not caused by AAT deficiency. The most promising are the results of investigations using animal models of human diseases such as type-1 diabetes, organ transplantation, influenza and HIV infections, heart failure, Crohn's disease and connective tissue diseases. These findings form a solid basis for future human trials.

So far, clinical data have been obtained in cystic fibrosis, and only in proof of concept studies that show evidence of anti-inflammatory effects in the lung. Trials in type-1 diabetes are under way; the results of these studies will be of important in shaping the future development of new AAT products given the high prevalence of type-1 diabetes. Other new indications for AAT therapy, especially in organ transplantation could also be addressed with clinical trials, but the expectations may be lower in these conditions.

With respect to new AAT formulations, aerosol AAT clearly is the closest to becoming clinically available for patients with AAT deficiency, cystic fibrosis and potentially COPD not associated with AAT deficiency. Significant challenges remain for the development of recombinant or transgenic AAT as a welcome substitute for human plasma-derived AAT, the only product currently available for clinical use. However, one is encouraged by the interest of the biologics industry finding new sources of AAT.

The regulatory approval for bringing a new AAT product to market and licensing it for new clinical uses is the final challenge to be faced going forward. However, the information presented at the workshop provided a firm basis for future human trials, some of which are likely to show that AAT can be considered a therapeutic agent in several diseases that are not caused by AAT deficiency.

Meeting participants

Co-chairs: Mark Brantly (University of Florida) Basil Golding (FDA-CBER)
Presenters: Eli Lewis (Diabetes, organ transplant/cell rejection)
Gerry McElvaney (cystic fibrosis, COPD)
Leland Shapiro (viral infection)
Sihong Song (connective tissue disease)
Charles Dinarello (diabetes, cardiovascular disease)
Lorraine Martin (cystic fibrosis, COPD)
Pnina Strauss (cystic fibrosis, bronchiectasis)
Marion Wencker (aerosol alpha-1 antitrypsin)
Mark Forshag (recombinant/transgenic alpha-1 antitrypsin)
Basil Golding (regulatory issues)

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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