Protease inhibitor therapeutics for respiratory disease

Disruption of the natural equilibria between proteases and their cognate inhibitors is a common feature of inflammatory disease. When this occurs in the lung, the effects can lead to irreversible impairment of pulmonary function. Although protease inhibition has been standard therapy for hereditary emphysema patients for many years, it is only recent studies that have predicted a further and more broad-based role for protease inhibitors in the treatment of respiratory disease. In contrast to current anti-inflammatory respiratory therapeutics, certain small molecule and protein protease inhibitors also have the capacity to inhibit directly the chronic airway remodelling and lung degeneration mediated by uncontrolled proteolytic activity. High efficiency delivery of such agents to the lung, therefore, will not only have a positive impact on lung inflammation, but will also have disease-modifying effects on the progressive loss of lung function caused by chronic degradation of lung tissue.

By Dr Philip J. Barr

To many, the age of blockbuster protease inhibitor therapeutics was heralded by the approval of aspartyl protease inhibitors for the treatment of HIV infection. Indeed, this major success story of the pharmaceutical industry has led to multiple protease inhibitor programmes geared towards the development of novel protease inhibitors that target further viral proteolytic enzymes, such as the NS3 protease of the hepatitis C virus (HCV), and the rhinovirus, or common cold virus 3C protease. Prior to these more recent developments, however, protease inhibitors were already in common usage for the treatment of hypertension and congestive heart failure and, by virtue of sales in the $ billions per annum during the 1980s, these angiotensin-converting enzyme (ACE) inhibitors were the blockbuster drugs of their time. This class of drugs continues to command enormous revenues to this day.

Shown in **Table 1** are the key members of the large families of approved aspartyl protease inhibitors that comprise the anti-hypertensive and anti-HIV therapeutics[1]. Despite the proven market potential of protease inhibitors, outside of the above two categories, the list of FDA-approved protease inhibitors is a short list indeed. As **Table 1** also shows, the only other protease inhibitors that are FDA-approved for human use are the protein therapeutic serine protease inhibitors, Trasylol, used in heart bypass surgery, and then a growing family of plasma-derived human neutrophil elastase (HNE) inhibitors. This latter class of inhibitors, Prolastin®, Aralast™ and Zemaira™ are plasma-derived alpha 1-antitrypsin

Table 1: FDA-Approved protease inhibitors; their protease targets and therapeutic indications (in chronological order of first approval of member of drug class)

TARGET PROTEASE	INDICATION	FDA-APPROVED INHIBITORS
Angiotensin-converting enzyme (ACE)	Hypertension, congestive heart failure	captopril/Capoten® (1981; Bristol-Myers Squibb) enalapril/enalaprilat/Vasotek® (Merck) lisinopril/Zestril® (AstraZeneca) benazepril/Lotensin® (Novartis) moexipril/Uniretic™/Univasc® (Schwarz Pharma) trandolapril/Mavik™ (Knoll) fosinopril/Monopril® (Bristol-Myers Squibb) ramipril/Altace® (Hoechst) quinapril/Accupril® (Parke-Davis)
Neutrophil elastase	Hereditary Emphysema	Prolastin® (1987; Bayer) Aralast™ (Baxter) Zemaira™ (Aventis Behring)
Kallikrein, plasmin	Heart bypass surgery	Aprotinin/Trasylol® (1993; Bayer)
HIV-1 protease	AIDS	saquinavir/Fortovase® (1995; Roche) ritonavir/Norvir® (Abbott) indinavir/Crixivan® (Merck) nelfinavir/Viracept® (Pfizer/Agouron) amprenavir/Agenerase® (GlaxoSmithKline)

(pAAT) replacement therapies for the treatment of the hereditary form of emphysema caused by AAT deficiency, with both Aralast™ and Zemaira™ each receiving FDA approval in 2003. Hereditary emphysema (HE) is a form of chronic obstructive pulmonary disease (COPD) that develops as a consequence of a deficiency of AAT in the circulation. The approval of these new pAAT therapeutics has

Table 2: Protease involvement in respiratory disease

RESPIRATORY DISEASE	PROTEASE(S) INVOLVED IN DISEASE ETIOLOGY AND PROGRESSION	REFERENCE(S)
Neonatal respiratory distress syndrome Chronic lung disease of prematurity	Neutrophil elastase	2,3
Airway hyperresponsiveness Asthma	Tissue kallikrein Mast cell chymase Mast cell tryptase Cathepsins ADAM33	4 5 6 7 8
Cystic fibrosis	Neutrophil elastase	9
Hereditary emphysema	Neutrophil elastase	10
COPD/Chronic bronchitis	Neutrophil elastase Matrix metalloproteases (1,2,8,9,12) TNF-α converting enzyme (TACE)	11 7,12-15 16

validated the utility of the class and may portend a shift in the way that not only HE, but other forms of COPD are treated. This article outlines how proteases are involved in virtually all respiratory diseases studied to date and, consequently, why lung disease, and particularly COPD, represents a huge but largely untapped market for protease inhibitor therapeutics.

Respiratory disease: a large and growing market

Respiratory disease is a significant cause of morbidity and mortality in individuals of all ages. From the neonate to the senior citizen, respiratory diseases can have severe acute manifestations or can involve long-term chronic symptoms, each of which can severely and adversely affect lung function. Indeed, many lung diseases are of such potential severity that they can lead, in some instances, to the premature death of the affected individual. For example, in the neonate, infant and young adult, respiratory distress syndrome (RDS), cystic fibrosis and, in rarer cases, asthma are potentially fatal diseases. In the higher age groups, the debilitating effects of chronic obstructive pulmonary disease (COPD) and chronic bronchitis represent significantly higher causes of morbidity and mortality. The National Heart, Lung and Blood Institute has reported that, with around 119,000 adults of ages 25 and older dying of COPD in 2000, this disease represents the fourth leading cause of death in the US

Of the indications that can likely be impacted by protease inhibitors (**Table 2**), the overlapping families of symptoms caused by asthma, COPD and chronic bronchitis are clearly the most prevalent (**Figure 1**), with many tens of millions of individuals affected by one or more of these disorders. Recent drug launches for the treatment of asthma have been blockbusters. For example, Merck and Co's launch of the orally active leukotriene receptor antagonist Singulair® in 2000 led to first year sales of more than $860 million. This tremendously successful product launch was actually eclipsed by GlaxoSmithKline's Advair®, an inhalable steroid and long-acting bronchodilator combination product. In its first eight months of sales, Advair® netted more than $1.2 billion in sales. This remarkably successful respiratory therapeutic was also approved in 2003 for the treatment of COPD and, along with Boehringer Ingelheim/Pfizer's Spiriva®, a once-a-day long-acting M3 muscarinic receptor antagonist, represents the immediate future of COPD therapy. Each of these drugs will likely each achieve sales in the $ billions for the treatment of COPD alone over the coming years.

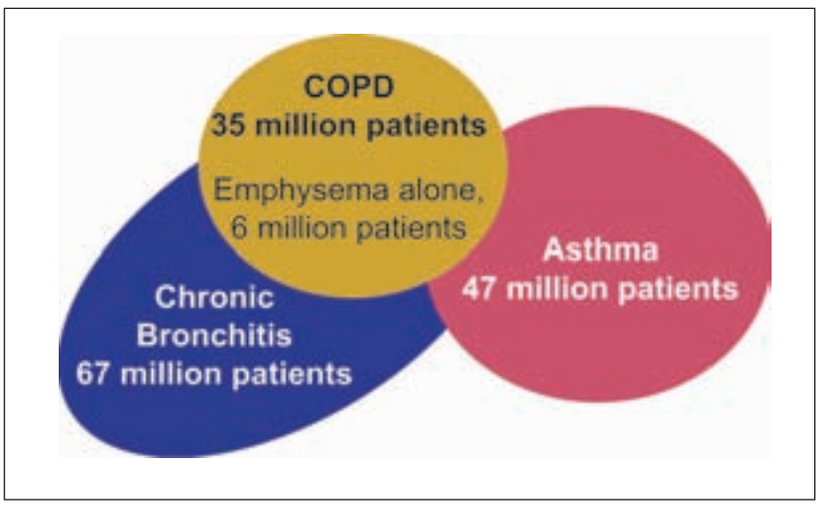

Figure 1
Disease prevalence for asthma and COPD is 5-7% of the population. Current market growth is 10% for asthma and 20% for COPD. Seven markets: France, Germany, Italy, Japan, Spain, UK, USA

Despite this, however, neither Advair® nor Spiriva® address the underlying pathology inherent in COPD. The eosinophilic inflammation in asthma is markedly suppressed by corticosteroids, but they have no appreciable effects on the inflammation in COPD, consistent with the failure of long-term corticosteroids to alter the progression of COPD[14]. Similarly, bronchodilation provides substantial palliative relief of symptoms, but does not address the long-term destruction of lung tissue arising from the protease/protease inhibitor imbalances, the chronic inflammation and the frequent exacerbations seen in COPD.

Proteases and protease inhibitors in respiratory disease

In asthma, members of the cysteinyl protease family of cathepsins have been implicated[7]. Also, more recent human genetic studies have shown a membrane-anchored metalloprotease, ADAM33, to be associated with asthma and bronchial hyperresponsiveness and therefore to be a novel therapeutic target[8,17]. To date, however, the main thrust of protease inhibitors for the treatment of asthma has been towards mast cell proteases, and particularly tryptase[18]. Mast cell chymase and tryptase are each known to contribute to airway inflammation in asthma. Tryptase also induces the proliferation of human airway smooth muscle cells through activation of the tethered-ligand G-protein-coupled receptor PAR-2 (protease-activated receptor-2)[19]. Accordingly, this target has come under intense scrutiny as a target for protease inhibition therapy, with specific inhibitors reaching Phase II human clinical trials.

In contrast to asthma, where morbidity is huge but mortality is comparatively rare (approximately 5,000 individuals succumb to severe asthma per year

Figure 2
AAT deficiency leads to an imbalance in the control of neutrophil elastase in the lung. In the unaffected individual (left), AAT is synthesised and secreted from the liver at high levels. In the majority of individuals with HE, a single point mutation in the AAT gene leads to the synthesis of a protein that polymerises during secretion from the liver. Only a small fraction of the mutant form of AAT is secreted into the bloodstream in these individuals

in the US), COPD is a pulmonary disease in which both morbidity and mortality are enormous. To address COPD using protease inhibitors, it has been instructive to take advantage of the finding that some individuals who are deficient in circulating levels of AAT are predisposed to early onset emphysema, and that this condition is exacerbated significantly by cigarette smoking. Also, the finding of a clear role for matrix metalloproteases in the development of smoking-related emphysema, and that these metalloproteases, along with neutrophil elastase and their endogenous inhibitors are all intimately related with respect to the modulation of their activities, has verified the validity of each of these protease classes as targets for COPD therapeutics.

AAT deficiency as a paradigm for COPD therapy

AAT is an endogenous acute phase plasma protein that has multiple functions. Produced at high levels in the liver in unaffected individuals, AAT plays a critical role in protecting against protease-mediated tissue degradation during periods of inflammation. Genetic deficiencies that lead to decreased levels of circulating AAT are linked to the onset of a number of diseases, most prominent among them being HE. In HE, the pathology of the disease is linked to degradation of pulmonary tissue elastin, mediated by unrestricted human neutrophil elastase (HNE) activity. This ultimately results in decreased lung elasticity and the presentation of clinical disease (**Figure 2**).

A commercially available form of AAT fractionated and purified from pooled human plasma was approved in 1987 for chronic replacement therapy of individuals having congenital AAT deficiency with clinically demonstrable emphysema. This product, Prolastin®, Bayer, enjoyed market exclusivity for many years but, as mentioned earlier, was followed by two new plasma-derived AATs that were approved in 2003. Aralast™ and Zemaira™ are now also marketed for the treatment of HE. Despite the utility of these products, however, their availability still does not make up

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the shortfall between the current availability of Prolastin and the diagnosed population suffering from the consequences of AAT deficiency. Accordingly, a recombinant form of AAT (rAAT), manufactured in genetically-engineered yeast cells, is currently being tested as an inhaled therapeutic in HE patients[20].

Chronic obstructive pulmonary disease (COPD) is a group of degenerative lung diseases that usually develop after many years of assault on lung tissues from cigarette smoke or other toxins and particles that pollute the air. These environmental insults to the lung destroy the alveoli, that stretch as they transport oxygen from the air to the blood and then shrink as they force out carbon dioxide. As a result, the damaged lungs lose their elasticity, exhaling becomes severely compromised and they cannot effectively exchange trapped air with fresh air.

A potential role for AAT in the treatment of emphysema caused by cigarette smoking has been known for more than two decades. Ever since the elucidation of the molecular mechanism of hereditary emphysema, it has been generally accepted that AAT would exert protective effects against proteolytic lung degradation in COPD. Proponents of early hypotheses concerning smoking-related emphysema considered cigarette smoke as an agent that could directly oxidise, and thereby inactivate, natural AAT in the lung. More recently, it became clear that reduced levels of AAT activity in the lung were more likely related to the indirect oxidation of AAT through generation of reactive oxygen species by activated neutrophils. Furthermore, it has also been shown that the macrophage-derived metalloproteases can inactivate AAT by proteolytic cleavage. Macrophages are extremely prominent in the smoker's lung, typically reaching steady state levels five- to 10-fold higher than those seen in the non-smoker's lung. More recently, it has been demonstrated that macrophage-derived metalloproteases activate tumour necrosis factor alpha (TNF-α) further enabling the recruitment of neutrophils that are key to the development of cigarette smoke-induced emphysema via HNE-mediated degradation of lung elastin[16]. Thus, although there is a clear involvement of macrophages and macrophage-derived metalloproteases in smoking-induced emphysema, the neutrophil-derived protease HNE is still considered to be the key mediator of lung degradation in COPD.

The limited supply of AAT isolated from human plasma has not allowed efficacy studies in human COPD, nor would this expensive therapy be economically viable. Recently, however, Churg et al[21], showed that injected Prolastin, was capable of reducing lung degeneration by 63% over a six-month period in a mouse model system for cigarette smoke-induced emphysema. The availability of inhalable forms of the less expensive recombinant AAT and the results of its testing in HE patients will allow its evaluation in the more prevalent forms of COPD induced primarily by cigarette smoking.

Current developmental strategies that address COPD using protease inhibitors

There exists a large body of literature on the potential use of small molecule elastase inhibitors in respiratory disease. For an all-encompassing and recent review of this subject, see reference 22. Of the numerous such molecules that have been tested, only one has been approved to date. Sivelestat (ONO-5046) is currently used in Japan for the treatment of acute lung injury[22]. Although the potent elastase-inhibitory activity of this molecule has suggested its potential use in COPD, chronic application of this and other small molecule elastase inhibitors have suffered from toxicity issues when introduced into human clinical trials. The animal model study using Prolastin has, however, suggested strongly that AAT has the capacity to inhibit lung degeneration induced by cigarette smoke, even when delivered by IP injection. Thus it is likely that a recombinant form of AAT, when administered more effectively through a modern high efficiency delivery device, will have similarly positive effects, without the toxicity issues.

In a second approach, based on the molecular principles described above, animal model studies for smoking-related emphysema using MMPIs have been extremely encouraging. In two particularly important studies, Martin et al[15] and Selman et al[23] demonstrated that orally or subcutaneously administered broad-spectrum MMPIs were capable of reducing dramatically the lung degeneration in cigarette smoke-treated mice and guinea pigs respectively. Because of the potential toxicity issues of such therapeutic regimens in humans, a further significant advance was recently reported by Pemberton et al. In this study, it was shown that nebulised ilomastat, the prototypic broad-spectrum MMPI, was capable of inhibiting alveolar degeneration by 96% when administered by nebulisation. Very low doses were required to achieve this effect in mice that were treated daily with cigarette smoke over a six-month period[24]. Ilomastat has sub-nanomolar Kis against many of the MMPs known to be involved in the development of emphysema[12,13]. In human COPD, the main contributors to the excess metalloprotease load in the lung are generally believed to be MMP-9 and MMP-12, with MMP-12 (murine

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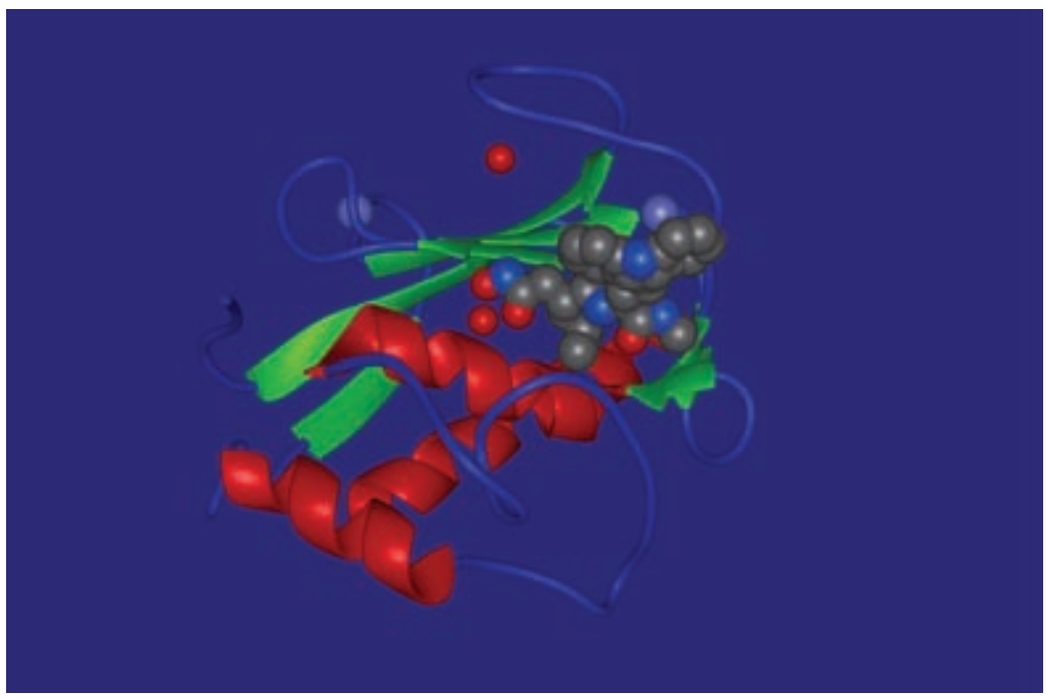

Figure 3
Space-filling model of the broad-spectrum MMPI, ilomastat, bound to the active site of human MMP-12

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macrophage elastase) also being a key factor in lung destruction in the smoke-treated mouse[13]. The ability of ilomastat to bind tightly at the active site of human MMP-12 has been shown by molecular modelling studies (**Figure 3**), based on the work described in reference 25. Further drug development work will be required to analyse the relative contributions of inhibition of each member of the MMP family towards amelioration of both direct extracellular matrix destruction and also towards blocking the activation of inflammatory proteins, such as TNF-α, that cause further direct lung tissue damage by recruitment of neutrophils, and generation of excessive HNE activity.

In conclusion, it is interesting to note that the finding that a blood deficiency of a natural protease inhibitor leads to early onset emphysema was first reported in 1963[26]. A protease inhibitor therapeutic, in the form of the plasma-derived Prolastin, was first approved to treat the underlying manifestations of the genetic form of emphysema in 1987, a gap of 24 years. Despite this approval, however, and because of the undersupply of the product, individuals suffering from other forms of chronic lung degeneration have had to endure another extremely barren period of disease-modifying drug discovery since then. The latest findings, described above, have shown that both recombinant protein and small molecule protease inhibitors are capable of disease-modifying effects in these additional chronic lung disorders. Emphysema patients can now look forward to the prospect of therapeutic protease inhibitors that will add to the current armamentarium of corticosteroids and bronchodilators by providing long-term protective effects over and above the symptom-treating drugs that are currently available. Therapies aimed towards sustained amelioration of the destruction of lung tissue architecture will likely have highly beneficial effects on oxygen transfer capacities, cardiovascular sequelae and other symptoms that are the ultimate causes of death in individuals suffering from severe respiratory disease. Therapeutic agents that can address these issues will generate substantial revenues that will also grow enormously as the population ages, and as the incidence of chronic degenerative lung disease increases. **DDW**

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