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Recent Advances in the Research and Development of Alpha-1 Proteinase Inhibitor for Therapeutic Use

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1. Introduction

Human alpha-1-proteinase inhibitor (α1-PI) is a well-characterized multifunctional protease inhibitor, the major physiological role of which is inhibition of neutrophil elastase (NE) in the lungs. The importance of α1-PI is underlined by its deficiency which is characterized by low levels of α1-PI in the circulation. Under such conditions, lower levels of α1-PI are transported to tissues, including the fragile alveoli of the lungs. α1-PI deficiency (with levels of α1-PI in blood below 11 μM, insufficient for inhibition of proteolytic enzymes in the lungs) is a common genetic condition predisposing α1-PI-deficient individuals to the development of chronic obstructive pulmonary disease (COPD). Hereditary α1-PI deficiency is classically associated with the development of premature, ultimately fatal, panacinar emphysema. To slow down the progression of emphysema, several licensed α1-PI concentrate preparations derived from pooled human plasma are currently available for intravenous augmentation therapy for patients with congenital α1-PI deficiency and clinically evident emphysema. In addition, and as an alternative to the plasma-derived α1-PI products, multiple efforts have been made to develop recombinant versions of human α1-PI over the last three decades. This review describes the recent advances in the research and development of human α1-PI for therapeutic use and covers the following: characterization of human α1-PI; epidemiology of α1-PI deficiency and currently licensed treatment; summary of the manufacturing and recent quality improvements of the α1-PI plasma-derived products; safety and efficacy of α1-PI intravenous augmentation and alternative routes; development of recombinant versions of human α1-PI; conditions other than emphysema that are associated with α1-PI; and some other aspects related to the research and development of α1-PI for therapeutic use.

* The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.
2. Human α₁-PI and α₁-PI deficiency

2.1 Structure and function of α₁-PI

Human alpha-1-proteinase inhibitor (α₁-PI), also known as alpha-1-antitrypsin, is the most abundant inhibitor of serine proteases in plasma. It is predominantly synthesized in hepatocytes, but is also produced, to a lesser extent, by alveolar macrophages, neutrophils, and some other cells (White et al., 1981; Carlson et al., 1988; Paakko et al., 1996). In healthy individuals, the concentration of α₁-PI in blood normally varies from 20 µM to 53 µM (1.04-2.76 g/L) (Brantly et al., 1988; Brantly et al., 1991) with a half-life in the circulation of about 3-5 days (Crystal, 1989; Kalsheker et al., 2002). Though α₁-PI has a wide range of inhibitory activities, its main physiological role is known to be the inhibition of polymorphonuclear leukocyte (neutrophil) elastase (NE) in the lungs (Travis, 1988). In the lower respiratory tract of healthy lungs, α₁-PI provides more than 90% of the anti-neutrophil elastase protection (Crystal, 1991; Crystal et al., 1989). Hereditary α₁-PI deficiency (with levels of α₁-PI in blood below 11 µM, insufficient for inhibition of NE) is classically associated with development of early-onset pulmonary emphysema, a hallmark of α₁-PI deficiency (Crystal et al., 1989; Snider, 1992). Smoking is known to be the biggest risk factor for developing emphysema; in smokers with α₁-PI deficiency a severe lung impairment is usually observed in their fourth decade of life.

α₁-PI is encoded by a single 12.2 kb gene (Pi) located on the long arm of chromosome 14 (Long et al., 1984; Rabin et al., 1986). Over 120 alleles of α₁-PI have been identified with approximately 35 of them being associated with α₁-PI deficiency, including Z-allele, which is the most common cause of the deficiency when inherited in a homozygous fashion. Due to a single mutation in the mobile domain (Glu342Lys), the α₁-PI Z-mutant undergoes aberrant conformational transitions that prompts the protein to aggregate. This results in retention of polymerized α₁-PI Z mutant within hepatocytes, thus inducing disease conditions in the liver and causing α₁-PI deficiency in the circulation (Ekeowa et al., 2011; Lomas, 2005; Volpert et al., 2000). The prevalence of three major α₁-PI variants (PiM, PiS, and PiZ) defines the number of carriers (PiMZ and PiMS) and individuals with deficiency phenotypes (PiZZ, PiSZ, and PiSS). The epidemiology of α₁-PI deficiency and its clinical manifestations, including lung diseases and liver diseases, has been described in detail (Ekeowa et al., 2011; Luiseiti & Seersholm, 2004; Needham & Stockley, 2004; Gooptu & Lomas, 2009). Based on the α₁-PI serum concentration, a common classification to define α₁-PI deficiency includes the four major categories: (1) normal (with α₁-PI serum levels not lower than 20 µM); (2) deficient (with α₁-PI concentrations in serum lower than 20 µM); (3) dysfunctional (with normal α₁-PI level, but lost or lower inhibitory activity); and (4) null (with α₁-PI serum concentrations below the detectable level).

α₁-PI is a 52 kDa glycoprotein belonging to the serine protease inhibitor (serpin) superfamily, in which addition to α₁-PI also includes α₁-antichymotrypsin, antithrombin, plasminogen activator inhibitor, C1 esterase inhibitor, and many others (Stein & Carrell, 1995; Silverman et al., 2001). A single polypeptide chain of α₁-PI is comprised of 394 amino acid residues, including one cysteine, 2 tryptophanes, and 9 methionine residues (Carp et al., 1982; Johnson & Travis, 1979). Three N-linked glycans attached to asparagine residues 46, 83, and 247 represent ~12% of α₁-PI by molecular weight (Mega et al., 1980a,b; Carrell et al., 1981, 1982). The carbohydrate moiety is comprised of biantennary N-glycans, but also triantennary and traces of tetraantennary structures grounded on the mannose fork core and containing N-acetyl glucosamine, galactose, and terminal negatively-charged sialic
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(N-acetylneuraminic) acid (Mega et al., 1980b; Travis & Salvesen, 1983; Kolarich et al., 2006a). The glycosylation pattern is a major cause of the iso-electric focusing (IEF) pattern typical for $\alpha_1$-PI with major isoforms M2, M4, M6, and also M7 and M8 due to the N-terminal truncation (Jeppsson et al., 1985; Kolarich et al., 2006a,b). Some characteristics of human $\alpha_1$-PI are listed in Table 1. Like the majority of other native glycoproteins, $\alpha_1$-PI is intrinsically a highly heterogeneous moiety, mainly due to variably trimmed glycosylation and an N-terminal pentapeptide that can be absent (Hercz, 1985; Krasnewich et al., 1995; Vaughan et al., 1982).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synonyms</td>
<td>alpha-1-proteinase inhibitor, alpha-1-antitrypsin</td>
</tr>
<tr>
<td>Common abbreviations</td>
<td>$\alpha_1$-PI, alpha-1-PI, $\alpha_1$-AT, alpha-1-AT, A1AT, A1T, AT</td>
</tr>
<tr>
<td>Classification</td>
<td>Serine proteinase inhibitor (serpin)</td>
</tr>
<tr>
<td>Substrates</td>
<td>Neutrophil elastase, trypsin, chymotrypsin</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>52,000 Da (50,300 Da by mass spectrometric analysis)</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>Three N-attached carbohydrates (12% w/w)</td>
</tr>
<tr>
<td>Polypeptide</td>
<td>Single polypeptide chain of 394 amino acid residues</td>
</tr>
<tr>
<td>Heterogeneity</td>
<td>Highly heterogeneous protein</td>
</tr>
<tr>
<td>Major isoforms</td>
<td>M2, M4, M6, M7 and M8</td>
</tr>
<tr>
<td>Half-life in circulation</td>
<td>3-5 days (for native plasma $\alpha_1$-PI)</td>
</tr>
<tr>
<td>Concentration in blood</td>
<td>Acute-phase plasma protein, concentration normally varies from 20 $\mu$M to 53 $\mu$M (1.04-2.76 g/L)</td>
</tr>
<tr>
<td>Major biological activities</td>
<td>Inhibitory anti-serine proteinase activity</td>
</tr>
<tr>
<td></td>
<td>Multiple non-inhibitory activities</td>
</tr>
<tr>
<td>Aggregation</td>
<td>$\alpha_1$-PI Z mutant is naturally prone to aggregation</td>
</tr>
<tr>
<td></td>
<td>$\alpha_1$-PI S mutant aggregates to a lower degree</td>
</tr>
<tr>
<td>Physiologically important phenotypes</td>
<td>PiMM (normal); PiSS, PiSZ &amp; PiZZ (deficiency phenotypes); PiZZ, PiSS &amp; PiNull (the most abnormal)</td>
</tr>
<tr>
<td>Diagnostic $\alpha_1$-PI variants</td>
<td>Normal (NLT$^a$ 20 $\mu$M); Deficient (lower than 20 $\mu$M; Dysfunctional (NLT 20 $\mu$M, inactive); Null (n.d.$^b$ level)</td>
</tr>
<tr>
<td>(serum concentrations)</td>
<td>Pulmonary and liver diseases</td>
</tr>
<tr>
<td></td>
<td>Other rare diseases (putative)$^c$</td>
</tr>
</tbody>
</table>

$^a$ NLT, not lower than; $^b$n.d., non-detectable; $^c$ See Table 3

Table 1. Characteristics of human $\alpha_1$-PI

Figure 1 shows a crystal structure of $\alpha_1$-PI, typical for serpins, which features 9 $\alpha$-helices, 3 $\beta$-sheets (A, B, and C), and a mobile 15-residue reactive center loop (RCL) exposed for interaction with the target serine protease (Johnson & Travis, 1979; Lomas, 2005). Protease attack of the RCL results in cleavage at Met358-Ser359, formation of a covalent $\alpha_1$-PI-protease complex with the amino-terminal polypeptide inserted into the A $\beta$-sheet, and an overall dramatic conformational change (Huntington et al., 2000; Ludeman et al., 2001; Stratikos & Gettins, 1999; Wilczynska et al., 1997).

Unlike the majority of proteins, $\alpha_1$-PI is naturally folded in a metastable structure which is essential for its function. This is not the most thermodynamically stable form, and thus, $\alpha_1$-PI is prone to a variety of conformational transitions and modifications (Lomas, 2005; Lomas...
et al., 1995). Much like other serpins, $\alpha_1$-PI can intramolecularly convert into a more stable latent form, which is inactive, but the biological activity can be restored via denaturation and refolding (Lomas et al., 1995; Silverman et al., 2001).

In addition to its inhibitory antiprotease function, $\alpha_1$-PI exhibits a broad spectrum of non-inhibitory activities (Brantly, 2002; Janciauskiene et al., 2011; Nita et al., 2005). Because of the nine methionine residues in $\alpha_1$-PI molecule, its plausible role as a putative antioxidant has been suggested (e.g., Levine et al., 1999, 2000).

Due to the abundance of $\alpha_1$-PI in human plasma and its conservative tertiary structure with hydrophobic cavities (Elliott et al., 2000; Lee et al., 2001; Parfrey et al., 2003), $\alpha_1$-PI has the capacity to bind small hydrophobic molecules. This property has been explored mainly with respect to the peptides and small molecules that may prevent the aggregation of the $\alpha_1$-PI Z mutant (Mahadeva et al. 2002; Mallya et al., 2007; Chang et al. 2009).

2.2 The $\alpha_1$-PI deficiency and $\alpha_1$-PI replacement therapy

There are approximately 60,000-100,000 severely deficient individuals in the United States which define $\alpha_1$-PI deficiency as a rare disease. However, according to several publications, $\alpha_1$-PI deficiency is widely under- and mis-diagnosed (e.g., de Serres, 2003; Bals et al., 2007). As reported by the World Health Organization (WHO, 1997), only 4% of the individuals with $\alpha_1$-PI deficiency cases are identified, and only a portion of them are receiving treatment. Currently licensed treatment of the patients with $\alpha_1$-PI deficiency and manifestation of pulmonary emphysema involves intravenous infusion of plasma-derived $\alpha_1$-PI preparations with the recommended dose of 60 mg of active $\alpha_1$-PI per kg of body weight administered once weekly. To maintain a threshold level of $\alpha_1$-PI (11µM), $\alpha_1$-PI-deficient patients should receive augmentation therapy for the duration of their lives, to slow the progression of emphysema. This nadir level has been determined based on $\alpha_1$-PI
levels observed in the plasma of individuals who are heterozygous for Z-mutant α₁-PI and who do not develop emphysema. Evaluation of the efficacy of α₁-PI products used in clinical studies is based on surrogate markers: the infusion of α₁-PI must elevate the circulating serum level of α₁-PI above an epidemiologically established ‘protective threshold’ and the protein must be detectable in bronchoalveolar lavage fluid (Juvellekian & Stoller, 2004; Sandhaus, 2009). However, the ability of α₁-PI augmentation therapy to reduce the progression of emphysema still remains to be proven. Safety and efficacy of intravenous α₁-PI augmentation are considered in section 3.3.1. For other disease conditions that may possibly benefit from α₁-PI therapy see section 3.3.3.

3. Research and development of α₁-PI for therapeutic use

3.1 Plasma-derived α₁-PI products

3.1.1 Currently approved α₁-PI products

Currently there are six commercial plasma-derived α₁-PI products (Table 2) licensed by the US FDA for intravenous treatment of patients with hereditary α₁-PI deficiency who show evidence of emphysema. Prolastin® (registered trade name of Bayer Corporation since 1987) was the first α₁-PI product to be approved. Since 2005, when Bayer Corporation was acquired by Talecris Biotherapeutics (Research Triangle Park, NC, USA; www.talecris.com), the product has been manufactured by Talecris. Aralast® (initially registered trademark of Alpha Therapeutic Corporation) was approved in 2003, and has been manufactured under the direction of Baxter Healthcare Corporation since then (Baxter, Westlake Village, CA, USA www.baxter.com). Zemaira® (registered trade name of Aventis Behring since 2003), another available product, is now manufactured by CSL Behring LLC (Kankakee, IL, USA; www.cslehring-us.com). In 2007, the US FDA approved another of Baxter’s preparations of α₁-PI concentrate - Aralast NP® - that has the same formulation as its predecessor, but differs from the earlier approved product by having a significantly lower content of C-terminal lysine-truncated α₁-PI (approximately 2% vs. 67%). In 2009, the US FDA approved Prolastin C®, the updated version of the earlier Talecris product that had been on the market for more than two decades. Due to more sophisticated purification and pathogen reduction steps, including two dedicated viral inactivation steps instead of heat treatment, the specific activity of Prolastin C® (above 0.7 mg of functional α₁-PI per mg of total protein) is twice higher than that of Prolastin®, which means that lower volumes and shorter transfusion time are needed. Most recently, in July 2010, the FDA approved Glassia™ (formerly Respira), a product manufactured by Kamada (Weizmann Science Park, Ness Ziona, Israel; www.kamada.com) and commercially launched by Baxter in the United States and some other countries. Glassia™ is another highly purified α₁-PI (with specific activity above 0.7 mg of active α₁-PI per mg of total protein) and the only α₁-PI product that is available in a ready-to-use liquid form with a shelf-life stability of two years under refrigerated conditions.

α₁-PI products are manufactured as part of a complex plasma fractionation scheme which was originally developed for large-scale production of albumin, but now also yields many other plasma therapeutics*. Since products are made from pooled human plasma, they may

* The US FDA product approval information is available at http://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProductsLicensedProductsBLAs/FractionatedPlasmaProducts/default.htm
Table 2. The plasma-derived α1-PI therapeutic products approved by the US FDA for chronic augmentation and maintenance therapy in adults with congenital α1-PI deficiency and clinically evident emphysema

<table>
<thead>
<tr>
<th>Drug product</th>
<th>Manufacturer</th>
<th>Date of licensure</th>
<th>Product form</th>
<th>Major steps of viral inactivation/removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolastin®</td>
<td>Talecris Biotherapeutics</td>
<td>12/2/1987</td>
<td>Lyophilized powder</td>
<td>Depth Filtration Heat Treatment</td>
</tr>
<tr>
<td>Aralast®c</td>
<td>Baxter Healthcare Co.</td>
<td>3/21/2003</td>
<td>Lyophilized powder</td>
<td>Solvent/Detergent &amp; Nanofiltration</td>
</tr>
<tr>
<td>Zemaira®</td>
<td>CSL Behring</td>
<td>7/8/2003</td>
<td>Lyophilized powder</td>
<td>Heat Treatment &amp; Ultrafiltration</td>
</tr>
<tr>
<td>Prolastin C®</td>
<td>Talecris Biotherapeutics</td>
<td>10/16/2009</td>
<td>Lyophilized powder</td>
<td>Solvent/Detergent &amp; Nanofiltration</td>
</tr>
<tr>
<td>Glassia™</td>
<td>Kamada</td>
<td>7/1/2010</td>
<td>Ready-to-use liquid</td>
<td>Solvent/Detergent &amp; Nanofiltration</td>
</tr>
</tbody>
</table>

*Based on recent publications including (Stockley, 2010; Tonelli & Brantly, 2010)

Reconstitution using Sterile Water for Injection is required

Aralast®, previously known as Respitin, contains approximately 67% of α1-PI with the truncated C-terminal lysine (Lys394)

Aralast NP® contains approximately 2% of α1-PI with truncation of C-terminal lysine residue

Heterogeneity of α1-PI products is a complex phenomenon. First of all, heterogeneous nature of plasma α1-PI is an intrinsic property of the native glycoprotein (see 2.1). Second, the presence of variously processed α1-PI forms including latent, cleaved, complexed or aggregated α1-PI species, is barely avoidable. However, it must be kept minimal as the inactive protein species have a direct influence on the product’s specific activity. Third, α1-PI products purified from pooled human plasma contain certain impurities of other plasma proteins, including albumin, haptoglobin, α1-antichymotrypsin, α1-lipoprotein, antithrombin III, C1-esterase inhibitor, etc. The human origin of these...
impurities ensures their tolerability, however, the level of these plasma proteins in α₁-PI concentrate may significantly increase the non-therapeutic protein load in the α₁-PI preparation intended for transfusion. In addition to all that, multistep manufacturing procedures are known to induce various protein alterations, such as aggregation and chemical modifications (e.g., deamidation, cysteinylation, and C-terminal truncation). Some modifications can be observed by IEF and other techniques (Cowden et al., 2005; Kolarich et al., 2006a, 2006b) and reflected in the product specifications. Currently there are no data that would demonstrate whether these alterations affect the in vivo activity, safety, efficacy or immunogenicity of α₁-PI therapeutic preparations. In general, commercial plasma-derived α₁-PI products differ in terms of their purity, specific activity, modifications, and excipients (Lomas et al., 1997; Cowden et al., 2005; Stockley, 2010; Tonelli & Brantly, 2010).

3.2 Research and development of the recombinant versions of human α₁-PI

3.2.1 Advances in the development of recombinant α₁-PI

The plasma supply per se is a limited source and appears to be insufficient to meet anticipated clinical demand. Moreover, despite effective viral inactivation/removal steps in the manufacturing of plasma proteins (Cai et al., 2005; Hotta et al., 2010), the risk of contamination with new and unknown pathogens may still exist. Therefore, recombinant technology has been widely explored as an alternative approach for the production of human α₁-PI since the pioneering works of the early 1980s (Bollen et al., 1983; Cabezon et al., 1984; Rosenberg et al., 1984). As evident from numerous reports, both from academic research and industry, the human gene for α₁-PI has been expressed in virtually all available hosts (E. coli, various yeasts, fungi, insect cells, CHO cells, human neuronal cells, and produced in transgenic plants and animals). For more details on research and development of recombinant α₁-PI (r-α₁-PI) in different systems and advances and limitations of the recombinant approach for production of stable and biologically active α₁-PI, see our comprehensive 2006 review (Karnaukhova et al., 2006). More recently, the human gene for α₁-PI has been expressed in filamentous fungi (Chill et al., 2009; Karnaukhova et al., 2007), transgenic tomato plants (Agarwal et al., 2009), tobacco cell cultures (Huang et al., 2009; Nadai et al., 2009), and human neuronal cell lines (Blanchard et al., 2011). Nevertheless, no r-α₁-PI is available as a licensed therapeutic treatment. In general, the essential criteria for the development of therapeutics for human use are safety, optimal clinical efficacy, and maximum cost-effectiveness. Among many efforts to develop r-α₁-PI of therapeutic quality (see Karnaukhova et al., 2006), there appear to be only two examples of the r-α₁-PLs for which development went far enough to get to clinical trials. The first was r-α₁-PI produced in the yeasts Saccharomyces cerevisiae and manufactured by Arriva Pharmaceuticals Inc. (Arriva) for several indications. A nebulized formulation of this non-glycosylated r-α₁-PI preparation has been intended for the treatment of respiratory disorders including emphysema and COPD (phase II clinical trials), and asthma (pre-clinical studies) (Brown, 2006a). Although animal studies have been considered to be successful (Pemberton et al., 2006), human trials have not been recommended (see review by Stokley, 2010). A topical gel formulation of r-α₁-PI has been intended for the treatment of dermatitis and other severe dermatological disorders in phase II clinical trials (see Brown, 2006b).

The second example of the advanced development of recombinant human α₁-PI is large scale production performed in transgenic dairy animals (t-α₁-PI): sheep [by PPL Therapeutics (UK) in partnership with Bayer Biologics (USA), (Dalrymple & Garner, 1998;
Wright et al., 1991), and goats [by Genzyme Transgenics Corporation (USA), (Ziomek, 1998)]. The transgenic α1-PI recovered from sheep milk was purified to 99.9% purity. Even so, sheep native α1-PI and sheep α1-antichymotrypsin were major impurities, at 6.7-18.7 mg/L and 60.3-75.8 parts per million, respectively. Two sequential clinical studies were performed to evaluate the safety and immunogenicity of aerosolized transgenic human α1-PI. None of the subjects had an antibody response to human r-α1-PI (Tebbutt, 2000; Spencer et al., 2005); however, antibody responses were observed to sheep α1-PI and to sheep α1-antichymotrypsin (Spencer et al., 2005). Four patients withdrew from the study due to the development of dyspnea and a decline in lung function, and the later product development was terminated.

3.2.2 Pitfalls in the development of r-α1-PI for therapeutic use
The general regulatory requirements for biologicals intended for therapeutic use, including r-α1-PI, are purity, safety, and efficacy. In order to be effective, therapeutic proteins have to be stable in vivo and in vitro (Karnaukhova et al., 2006). Reviewing the work performed over the last two decades to produce stable and biologically active r-α1-PI of therapeutic quality, one can see basically two major factors that were impeding the progress: (1) impurities that could induce antibody responses and cause adverse reactions in patients, and (2) lower stability than that of plasma counterpart, mainly caused by the lack of glycosylation or non-human type of glycosylation (the latter may also induce immune responses). Although presently the first reason can be technically better solved, removal of trace amounts of non-human native proteins derived from the host, e.g., sheep α1-PI, from the human r-α1-PI to exclude further adverse reactions, requires a much higher level of purification than was possible at the time of that development. As for the second reason, indeed, glycosylation is considered to be a cause of rapid clearance of r-α1-PI from the circulation (Casolaro et al., 1987; Cantin et al., 2002a). Aberrant glycosylation (or lack of glycans) does not necessarily affect biological activity of the recombinant protein, but it is important for its stability. According to recently published data, glycosylation of α1-PI does not interfere with the serpin native state flexibility (or instability) essential for its efficient function, though it may confer resistance to degradation by proteases and thus extend its half-life in the circulation (Sarkar & Wintrode, 2011). Extensive work performed over decades for the development of viable r-α1-PI of therapeutic quality and lessons learned from these experiences truly paved the way for other protein therapeutics. It is worthwhile to mention two serpins produced in transgenic animals that were recently approved. In 2009, the US FDA approved recombinant antithrombin (ATryn®) produced in the milk of transgenic goats (Fyfe & Tait, 2009). In 2010, another serpin, recombinant human C1-esterase inhibitor (Ruconest®) produced in the milk of transgenic rabbits was granted European marketing authorization (Varga & Farkas, 2011). Both pharmaceutical proteins show a faster clearance, yet it may not be an issue depending on the intended use. For instance, Ruconest® was approved for the treatment of acute attacks of hereditary angioedema, and therefore there is no need to maintain its higher level in blood longer than its action is required. Given a shorter in vivo half-life of recombinant α1-PI, it has been considered for other administration routes and applications, such as inhalation for the treatment of emphysematous condition, and topical application for various skin diseases. However, a convincing proof of the recombinant product efficacy and safety in appropriate clinical trials is as problematic as it is for plasma-derived α1-PI; large clinical trials in the cases of rare diseases are difficult to perform because of small geographically dispersed patient populations. In addition, a limited population means a
limited market, which is less attractive for large investments. No doubt, these reasons markedly slow down the development of r-α1-PI.

3.3 α1-PI–based therapies
3.3.1 Safety and efficacy of intravenous α1-PI augmentation
The intravenous augmentation of α1-PI was shown to be safe and well tolerated over a long history of the replacement therapy. However, its impact on disease progression and mortality still remains to be convincingly proven. α1-PI augmentation is assumed to slow down the rate of emphysema development and progression, and thus, to improve the life quality and duration of α1-PI deficient patients, yet the essential proof of efficacy is missing. According to Hubbard & Crystal (1990), only approximately 2-3% of infused α1–PI actually reaches the lungs; and the effectiveness of α1-PI replacement therapy has been evaluated mainly on the bases of biochemical (not clinical) criteria (Tonelli & Brantly, 2010). For recently approved α1-PI products, their pharmacokinetic equivalence and comparable safety profile to Prolastin were demonstrated (e.g., Stocks et al., 2010). α1-PI therapy is a life-long and very expensive treatment that may cost up to $150,000 (Silverman, 2009) in the United States. Whether this therapy decreases mortality also remains unknown, as there are no reliable data on mortality, as well as morbidity and survival (Gøtzsche & Johansen, 2010a). Some observational studies support the idea that augmentation therapy may help to slow the decline in lung function (Seersholm et al., 1997; Wencker et al., 2001; Kueppers, 2011). But there are also more critical evaluations including the opinion that α1-PI augmentation therapy cannot be recommended due to lack of evidence of clinical benefit and the cost of treatment (Gøtzsche & Johansen, 2010a, 2010b). It is currently widely admitted that the efficacy of α1-PI augmentation therapy has never been persuasively demonstrated and must be proven in a proper clinical trial. Due to the widespread and small clusters of patients all over the country, conducting a prospective, randomized, placebo-controlled clinical trial is challenging. In addition, the development of emphysema proceeds slowly, creating the additional difficulties of monitoring lung function decline and mortality data (Hutchinson & Hughes, 1997; Schluchter et al., 2000).

3.3.2 Alternative routes of administration of α1-PI products
Due to the inconvenience of life-time intravenous augmentation therapy and low levels of α1–PI reaching lungs, the inhalation of aerosolized α1-PI has been suggested as a less invasive and more efficient way to deliver large amounts of α1-PI directly to the lungs where it is most needed (Hubbard et al., 1989; McElvaney et al., 1991; Cockett, 1999). Although strategies for aerosol therapy of α1-PI deficiency has been proposed two decades ago (Hubbard et al., 1989; Hubbard & Crystal, 1990), there is still no α1-PI aerosolized treatment approved. Several studies examined efficiency of the α1-PI inhalation therapy in animals and in humans (Kropp et al., 2001; Siekmeier, 2010). It was demonstrated (Kropp et al., 2001) that significantly more α1-PI was deposited in the lungs through the inhalational route than via intravenous infusion (14.6% vs. 2%). Although the inhalation route seems attractive, nevertheless, enabling the inhaled material to reach the lung interstitium, the most important to the emphysematous process region, is still problematic. With regards to recombinant versions of α1-PI, it is generally assumed that products directly delivered to the lungs may not require the same degree of stability as α1-PI given intravenously. However, as mentioned above, human studies using r-α1-PI from transgenic sheep were associated
with adverse reactions due to impurities derived from the host (Spenser et al., 2005). Thus, higher levels of purification and more clinical studies are required.

### 3.3.3 Other α₁-PI applications

Currently, α₁-PI therapeutic preparations are licensed exclusively for one indication, i.e., chronic augmentation and maintenance therapy in individuals with emphysema due to congenital α₁-PI deficiency. Previously unrecognized inherited disorder, α₁-PI deficiency was first described in 1963 (Laurell & Eriksson, 1963) based on the serum electrophoretic analysis that revealed five individuals deficient of α₁-fraction; three of those patients had developed emphysematous conditions. Six years later, in 1969, cirrhosis associated with α₁-PI deficiency was described (Sharp et al., 1969). These findings initiated a concept of linkage between α₁-PI deficiency and pulmonary and liver diseases. As evident from the available literature, due to the multiple biological activities of α₁-PI, it has been associated with other lung diseases (first of all, cystic fibrosis) and many non-pulmonary diseases (Table 3). Some of these conditions may possibly benefit from α₁-PI augmentation therapy (see recent reviews by Blanco et al., 2011 and Janciauskiene et al., 2011).

According to Blanco et al. (2011), α₁-PI therapy has proven remarkable efficacy in small cohorts of α₁-PI-deficient patients who also suffer from fibromyalgia, systemic vasculitis, relapsing panniculitis and bronchial asthma. Although the putative benefits of α₁-PI therapy for treatment of additional rare diseases (some are listed in Table 3) requires much more clinical data than are currently available to support clinical efficacy and safety of α₁-PI treatment, in general it indicates a clear potential for additional α₁-PI supply to satisfy the anticipated clinical demand in near future. Because of controversy related to the additional clinical implications of α₁-PI deficiency, more clinical data are needed to verify whether the reported links between α₁-PI deficiency and other rare diseases are real or accidental.

As a potent anti-inflammatory agent, α₁-PI has been investigated in clinical studies for treatment of cystic fibrosis (Jones & Helm, 2009). Whereas patients with emphysematous conditions suffer from the hereditary α₁-PI deficiency and, thus, insufficient levels of the protease inhibitor in the lungs due to impaired α₁-PI synthesis in hepatocytes, patients with cystic fibrosis may have normal synthesis of α₁-PI and suffer from severe pulmonary inflammation due to high excess of NE in the lungs, leading to a progressive loss of lung function (Allen, 1996; Siekmeier, 2010). Therefore, it has been proposed that both groups of patients may benefit from α₁-PI augmentation therapy to prevent the deleterious effect of free protease (Allen, 1996; Birrer, 1995; Birrer et al, 1996). However, intravenous administration of α₁-PI did not result in a suppression of the respiratory neutrophil elastase burden (McElvaney et al, 1991). Several studies have been conducted using inhalation of an aerosolized α₁-PI in cystic fibrosis and α₁-PI deficiency (Hubbard et al., 1989; Griese et al, 2001, 2007; Martin et al, 2006; Brand et al, 2009).

Whereas several studies that investigated the efficacy of treatment with an aerosolized α₁-PI both in patients with cystic fibrosis and in those with α₁-PI deficiency came to positive conclusions regarding deposition of inhaled α₁-PI in the lungs and its anti-elastase activity (see review by Siekmeier, 2010), the conclusion from other studies was that treatment with α₁-PI did not demonstrate any clinical improvements (Martin, 2006). If further clinical studies support the safety and efficacy of an aerosolized α₁-PI, and it is approved for treatment of cystic fibrosis, the demand for therapeutic α₁-PI preparations could be significantly increased.
### Table 3. Conditions other than emphysema and liver disease possibly associated with \( \alpha_1\)-PI

<table>
<thead>
<tr>
<th>Disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasculitis</td>
<td>Dowd et al., 1995; Esnault, 1997; Griffith et al., 1996</td>
</tr>
<tr>
<td>Panniculitis</td>
<td>Chowdhury et al., 2002; Gross et al., 2009; Kjus et al., 2002; Smith et al., 1987; Valverde et al., 2008</td>
</tr>
<tr>
<td>Fibromyalgia</td>
<td>Ablin et al., 2009; Blanco et al., 2004; Blanco et al., 2010</td>
</tr>
<tr>
<td>Asthma</td>
<td>Blanco et al., 2008; Blanco et al., 2011; Eden et al., 1997</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>Rabassa et al., 1995; Needham &amp; Stockley, 2004</td>
</tr>
<tr>
<td>Renal</td>
<td>Szönyi et al., 2006; Ting et., 2008</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Kalis et al., 2010; Lisowska-Myjak et al., 2006;</td>
</tr>
<tr>
<td>Cancer</td>
<td>Li et al., 2011; Lindor et al., 2010; Topic et al., 2011</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Grimstein et al., 2010; Grimstein et al., 2011</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>Stakisaitis et al., 2001; Talmud et al., 2003;</td>
</tr>
<tr>
<td>Acute anterior uveitis</td>
<td>Fearnley et al., 1988; Saari et al., 1986</td>
</tr>
<tr>
<td>Chronic rhinosinusitis</td>
<td>Kilty et al., 2008, 2010; Maune et al., 1995</td>
</tr>
</tbody>
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### 3.3.4 Research toward the enhancement of \( \alpha_1\)-PI-therapies

During last decade various approaches have been considered for the enhancement of \( \alpha_1\)-PI-based therapies. For instance, to prolong a short half-life of r-\( \alpha_1\)-PI in the circulation, Cantin and co-workers hypothesized that conjugation of r-\( \alpha_1\)-PI with polyethylene glycol (PEG) at Cys²³² could extend the *in vivo* half-life of recombinant protein in blood and lung (Cantin et al., 2002b). According to their data, the site-specific conjugation with either 20 or 40 kD PEG at Cys²³² of nonglycosylated r-\( \alpha_1\)-PI (human) results in an active inhibitor with extended *in vivo* stability. Moreover, 72 h later after airway instillation, the PEG-r-\( \alpha_1\)-PI seemed to be significantly better than glycosylated \( \alpha_1\)-PI at protecting the lung against elastase–induced lung hemorrhage. As an example of the *in vitro* biochemical evaluation of the concept, \( \alpha_1\)-PI has been considered for its affinity to various small ligands and drugs for different reasons. Mainly this approach has been explored with respect to the peptides and small molecules in order to prevent the aggregation of Z mutant (e.g., Mallya et al., 2007; Chang et al. 2009). In the meantime, the protein’s potential for binding small ligands of pharmaceutical interest has been proposed as a promising approach that is directed at, and may ultimately enhance, currently existing \( \alpha_1\)-PI therapies (Karnaukhova et al., 2010). For instance, \( \alpha_1\)-PI’s affinity to retinoic acid, which is known for a wide range of physiological activities including alveolar repair and regrowth (Roche clinical studies, see Stockley, 2010; Massaro & Massaro, 1996, 1997) and tissue rejuvenation in various dermatologic diseases, has been convincingly demonstrated in biochemical experiments *in vitro* (Karnaukhova et al., 2010). As \( \alpha_1\)-PI augmentation therapy cannot cure, but may only slow down, the progression of emphysema, its complexion with retinoic acid could be more efficient for treatment than \( \alpha_1\)-PI alone. It is noteworthy that the interactions of \( \alpha_1\)-PI with several other physiologically active ligands (including porphyrins) may reveal additional properties of this multifunctional serpin.

### 4. Conclusions

Since \( \alpha_1\)-PI deficiency was first described by Carl-Bertil Laurell and Sten Eriksson (Laurell & Eriksson, 1963) as a condition that could lead to the development of severe obstructive
pulmonary disease, our knowledge about $\alpha_1$-PI structure-function relationships and clinical manifestations of $\alpha_1$-PI deficiency has increased tremendously. Moreover, multi-disciplinary research efforts prompted the development of $\alpha_1$-PI-based augmentation therapy to maintain the inhibitor level above the protective threshold. Since 1987, several $\alpha_1$-PI products derived from pooled human plasma have been approved and are currently available to slow down the progression of emphysematous conditions in $\alpha_1$-PI-deficient patients. In addition, due to its multiple physiological activities, $\alpha_1$-PI has been identified for its putative involvement in several other rare diseases, the treatment of which may possibly benefit from $\alpha_1$-PI-based therapies. As an alternative to intravenous administration that may improve the efficacy of $\alpha_1$-PI treatment, the inhalation of aerosolized $\alpha_1$-PI preparations has been in clinical trials. Recombinant versions of human $\alpha_1$-PI have been produced in all available hosts and in several transgenic animals. These efforts made a remarkable impact on the research realm of recombinant protein therapeutics, but did not yet bring any viable version of recombinant $\alpha_1$-PI to the treatment. In regards to therapeutic preparations and their use, there are several questions to be addressed when looking to the future. Keeping in mind the long history of replacement therapy using currently approved plasma-derived $\alpha_1$-PI products, it is essential that the efficacy of $\alpha_1$-PI replacement therapy be clearly demonstrated in prospective, randomized, placebo-controlled trials. Will the efficacy of inhalation therapy using aerosolized $\alpha_1$-PI preparations be proven to be superior to that of the intravenous route? Will the recombinant/transgenic versions of human $\alpha_1$-PI be optimized to meet the requirements for protein therapeutics? Will other rare diseases currently implicated in association with $\alpha_1$-PI and $\alpha_1$-PI deficiency be clearly proven to benefit from $\alpha_1$-PI treatment? From the standpoint of product quality, safety and efficacy, the current state of research and development of $\alpha_1$-PI for therapeutic use demonstrates a symbiosis of the recent achievements and controversies, hopefully typical of our progress.

5. References


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The developments in molecular medicine are transforming respiratory medicine. Leading clinicians and scientists in the world have brought their knowledge and experience in their contributions to this book. Clinicians and researchers will learn about the most recent advances in a variety of lung diseases that will better enable them to understand respiratory disorders. This treatise presents state of the art essays on airways disease, neoplastic diseases, and pediatric respiratory conditions. Additionally, aspects of immune regulation, respiratory infections, acute lung injury/ARDS, pulmonary edema, functional evaluation in respiratory disorders, and a variety of other conditions are also discussed. The book will be invaluable to clinicians who keep up with the current concepts, improve their diagnostic skills, and understand potential new therapeutic applications in lung diseases, while scientists can contemplate a plethora of new research avenues for exploration.

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