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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 (α_1-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 α_1-PI is underlined by its deficiency which is characterized by low levels of α_1-PI in the circulation. Under such conditions, lower levels of α_1-PI are transported to tissues, including the fragile alveoli of the lungs. α_1-PI deficiency (with levels of α_1-PI in blood below 11 μM, insufficient for inhibition of proteolytic enzymes in the lungs) is a common genetic condition predisposing α_1-PI-deficient individuals to the development of chronic obstructive pulmonary disease (COPD). Hereditary α_1-PI deficiency is classically associated with the development of premature, ultimately fatal, panacinar emphysema. To slow down the progression of emphysema, several licensed α_1-PI concentrate preparations derived from pooled human plasma are currently available for intravenous augmentation therapy for patients with congenital α_1-PI deficiency and clinically evident emphysema. In addition, and as an alternative to the plasma-derived α_1-PI products, multiple efforts have been made to develop recombinant versions of human α_1-PI over the last three decades. This review describes the recent advances in the research and development of human α_1-PI for therapetic use and covers the following: characterization of human α_1-PI; epidemiology of α_1-PI deficiency and currently licensed treatment; summary of the manufacturing and recent quality improvements of the α_1-PI plasma-derived products; safety and efficacy of α_1-PI intravenous augmentation and alternative routes; development of recombinant versions of human α_1-PI; conditions other than emphysema that are associated with α_1-PI; and some other aspects related to the research and development of α_1-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 α_1-PI and α_1-PI deficiency

2.1 Structure and function of α_1-PI

Human alpha-1-proteinase inhibitor (α_1−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 lower 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 α_1−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 α_1−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, α_1–PI provides more than 90% of the anti–neutrophil elastase protection (Crystal, 1991; Crystal et al., 1989). Hereditary α_1−PI deficiency (with levels of α_1−PI in blood below 11 μM, insufficient for inhibition of NE) is classically associated with development of early-onset pulmonary emphysema, a hallmark of α_1−PI deficiency (Crystal et al., 1989; Snider, 1992). Smoking is known to be the biggest risk factor for developing emphysema; in smokers with α_1−PI deficiency a severe lung impairment is usually observed in their fourth decade of life.

α_1-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 α_1−PI have been identified with approximately 35 of them being associated with α_1−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 α_1−PI Z-mutant undergoes aberrant conformational transitions that prompts the protein to aggregate. This results in retention of polymerized α_1−PI Z mutant within hepatocytes, thus inducing disease conditions in the liver and causing α_1−PI deficiency in the circulation (Ekeowa et al., 2011; Lomas, 2005; Volpert et al., 2000). The prevalence of three major α_1−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 α_1−PI deficiency and its clinical manifestations, including lung diseases and liver diseases, has been described in detail (Ekeowa et al., 2011; Luisetti & Seersholm, 2004; Needham & Stockley, 2004; Gooptu & Lomas, 2009). Based on the α_1−PI serum concentration, a common classification to define α_1−PI deficiency includes the four major categories: (1) normal (with α_1−PI serum levels not lower than 20 μM); (2) deficient (with α_1−PI concentrations in serum lower than 20 μM); (3) dysfunctional (with normal α_1−PI level, but lost or lower inhibitory activity); and (4) null (with α_1−PI serum concentrations below the detectable level).

α_1−PI is a 52 kDa glycoprotein belonging to the serine protease inhibitor (serpin) superfamily, which in addition to α_1-PI also includes α_1-antichymotrypsin, antithrombin, plasminogen activator inhibitor, C1 esterase inhibitor, and many others (Stein & Carrell, 1995; Silverman et al., 2001). A single polypeptide chain of α_1-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 α_1−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

(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 α_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 α_1−PI are listed in Table 1. Like the majority of other native glycoproteins, α_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).

Characteristics	Description
Synonyms	alpha-1-proteinase inhibitor, alpha-1-antitrypsin
Common abbreviations	α_1−PI, alpha-1-PI, α_1−AT, alpha-1-AT, A1AT, ATT, AT
Classification	Serine proteinase inhibitor (serpin)
Substrates	Neutrophil elastase, trypsin, chymotrypsin
Molecular weight	52,000 Da (50,300 Da by mass spectrometric analysis)
Glycosylation	Three N-attached carbohydrates (12% w/w)
Polypeptide	Single polypeptide chain of 394 amino acid residues
Heterogeneity	Highly heterogeneous protein
Major isoforms	M2, M4, M6, M7 and M8
Half-life in circulation	3-5 days (for native plasma α_1−PI)
Concentration in blood	Acute-phase plasma protein, concentration normally varies from 20 μM to 53 μM (1.04-2.76 g/L)
Major biological activities	Inhibitory anti-serine proteinase activity Multiple non-inhibitory activities
Aggregation	α_1−PI Z mutant is naturally prone to aggregation α_1−PI S mutant aggregates to a lower degree
Physiologically important phenotypes	PiMM (normal); PiSS, PiSZ & PiZZ (deficiency phenotypes); PiZZ, PiSS & PiNull (the most abnormal)
Diagnostic α_1-PI variants (serum concentrations)	Normal (NLT[a] 20 μM); Deficient (lower than 20 μM; Dysfunctional (NLT 20 μM, inactive); Null (n.d.[b] level)
Diseases related to α_1−PI deficiency and aggregation	Pulmonary and liver diseases Other rare diseases (putative)[c]

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

Table 1. Characteristics of human α_1-PI

Figure 1 shows a crystal structure of α_1-PI, typical for serpins, which features 9 α-helices, 3 β-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 α_1-PI-protease complex with the amino-terminal polypeptide inserted into the A β-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, α_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, α_1-PI is prone to a variety of conformational transitions and modifications (Lomas, 2005; Lomas

et al., 1995). Much like other serpins, α_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).

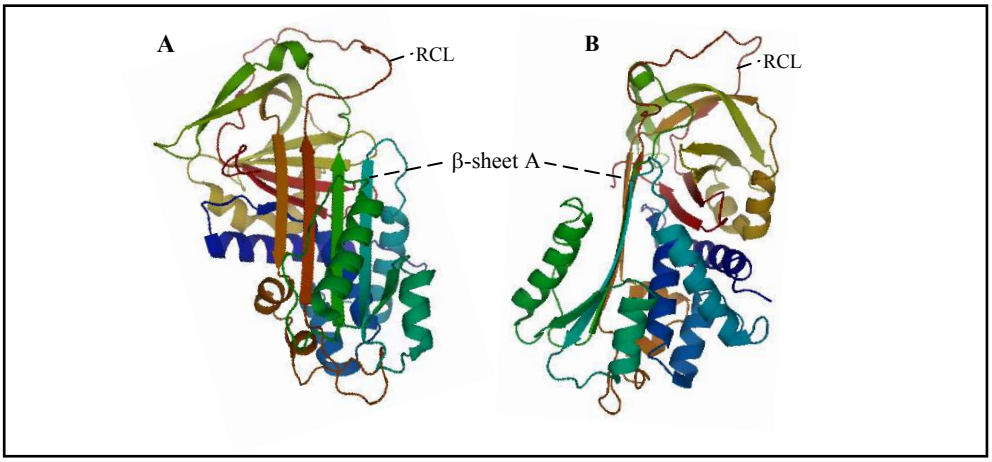

Fig. 1. Crystal structure of α_1-PI (PDB 1HP7) in two projections. (**A**) Front view at the α_1-PI structure in respect to β-sheet A, and (**B**) Side view obtained by 90° clockwise rotation of the molecule. The images were obtained using PyMOL (the PyMOL Molecular Graphics System, Version 1.1r1, Schrödinger, LLC).

In addition to its inhibitory antiprotease function, α_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 α_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 α_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), α_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 α_1−PI Z mutant (Mahadeva et al. 2002; Mallya et al., 2007; Chang et al. 2009).

2.2 The α_1-PI deficiency and α_1-PI replacement therapy

There are approximately 60,000-100,000 severely deficient individuals in the United States which define α_1-PI deficiency as a rare disease. However, according to several publications, α_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 α_1-PI deficiency cases are identified, and only a portion of them are receiving treatment. Currently licensed treatment of the patients with α_1-PI deficiency and manifestation of pulmonary emphysema involves intravenous infusion of plasma-derived α_1-PI preparations with the recommended dose of 60 mg of active α_1-PI per kg of body weight administered once weekly. To maintain a threshold level of α_1-PI (11μM), α_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 α_1-PI

levels observed in the plasma of individuals who are heterozygous for Z-mutant α_1−PI and who do not develop emphysema. Evaluation of the efficacy of α_1-PI products used in clinical studies is based on surrogate markers: the infusion of α_1-PI must elevate the circulating serum level of α_1−PI above an epidemiologically established 'protective threshold' and the protein must be detectable in bronchoalveolar lavage fluid (Juvelekian & Stoller, 2004; Sandhaus, 2009). However, the ability of α_1-PI augmentation therapy to reduce the progression of emphysema still remains to be proven. Safety and efficacy of intravenous α_1-PI augmentation are considered in section 3.3.1. For other disease conditions that may possibly benefit from α_1-PI therapy see section 3.3.3.

3. Research and development of α_1-PI for therapeutic use

3.1 Plasma-derived α_1-PI products

3.1.1 Currently approved α_1-PI products

Currently there are six commercial plasma-derived α_1-PI products (Table 2) licensed by the US FDA for intravenous treatment of patients with hereditary α_1-PI deficiency who show evidence of emphysema. Prolastin® (registered trade name of Bayer Corporation since 1987) was the first α_1-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.cslbehring-us.com). In 2007, the US FDA approved another of Baxter's preparations of α_1-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 α_1-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 α_1-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 α_1-PI (with specific activity above 0.7 mg of active α_1-PI per mg of total protein) and the only α_1-PI product that is available in a ready-to-use liquid form with a shelf-life stability of two years under refrigerated conditions.

α_1−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/ApprovedProducts/LicensedProductsBLAs/FractionatedPlasmaProducts/default.htm

Drug product	Manufacturer	Date of licensure	Product form	Major steps of viral inactivation/removal
Prolastin®	Talecris Biotherapeutics	12/2/1987	Lyophilized powder[b]	Depth Filtration Heat Treatment
Aralast®[c]	Baxter Healthcare Co.	3/21/2003	Lyophilized powder	Solvent/Detergent & Nanofiltration
Zemaira®	CSL Behring	7/8/2003	Lyophilized powder	Heat Treatment & Ultrafiltration
Aralast NP®[d]	Baxter Healthcare Co.	5/4/2007	Lyophilized powder	Solvent/Detergent & Nanofiltration
Prolastin C®	Talecris Biotherapeutics	10/16/20099	Lyophilized powder	Solvent/Detergent & Nanofiltration
Glassia™	Kamada	7/1/2010	Ready-to-use liquid	Solvent/Detergent & Nanofiltration

[a] Based on recent publications including (Stockley, 2010; Tonelli & Brantly, 2010)
[b] Reconstitution using Sterile Water for Injection is required
[c] Aralast®, previously known as Respitin, contains approximately 67% of α_1-PI with the truncated C-terminal lysine (Lys394)
[d] Aralast NP® contains approximately 2% of α_1-PI with truncation of C-terminal lysine residue

Table 2. The plasma-derived α_1-PI therapeutic products approved by the US FDA for chronic augmentation and maintenance therapy in adults with congenital α_1-PI deficiency and clinically evident emphysema[a]

carry the risk of transmitting human infectious agents, *e.g.*, some viruses, and theoretically, the Creutzfeldt-Jakob disease (CJD) agent or disease variant agents, as well as emerging or unknown infectious agents. To reduce the potential risk of transmitting infectious agents, the α_1-PI preparations are manufactured using a number of viral inactivation and removal steps. Currently approved α_1−PI products differ in the procedures used for pathogen reduction. For instance, the heat treatment procedure used in manufacturing of Prolastin was one of the reasons for higher content of inactive and aggregated α_1−PI in the product. The manufacturing procedures of the later products (Table 2) include two dedicated steps that are specifically designed for inactivation and removal of viruses (Hotta et al., 2010). Thus, solvent/detergent treatment and nanofiltration are used as the dedicated pathogen reduction steps in the manufacturing of recently approved Prolastin C®, Glassia™ and both Aralast products. Overall, the manufacturing history of plasma-derived α_1-PI therapeutic products reflects a trend of continous improvement of product quality.

3.1.2 Heterogeneity of α_1-PI products

Heterogeneity of α_1-PI therapeutic preparations is a complex phenomenon. First of all, heterogeneous nature of plasma α_1-PI is an intrinsic property of the native glycoproten (see 2.1). Second, the presence of variously processed α_1-PI forms including latent, cleaved, complexed or aggregated α_1-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, α_1-PI products purified from pooled human plasma contain certain impurities of other plasma proteins, including albumin, haptoglobin, α_1-antichymotrypsin, α_1-lipoprotein, antithrombin III, C1-esterase inhibitor, etc. The human origin of these

impurities ensures their tolerability, however, the level of these plasma proteins in α_1-PI concentrate may significantly increase the non-therapeutic protein load in the α_1-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 α_1-PI therapeutic preparations. In general, commercial plasma-derived α_1-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 α_1-PI

3.2.1 Advances in the development of recombinant α_1-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 α_1-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 α_1-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 α_1-PI (r-α_1-PI) in different systems and advances and limitations of the recombinant approach for production of stable and biologically active α_1-PI, see our comprehensive 2006 review (Karnaukhova et al., 2006). More recently, the human gene for α_1-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-α_1-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-α_1-PI of therapeutic quality (see Karnaukhova et al., 2006), there appear to be only two examples of the r-α_1-PIs for which development went far enough to get to clinical trials. The first was r-α_1-PI produced in the yeasts *Saccromyces cerevisiae* and manufactured by Arriva Pharmaceuticals Inc. (Arriva) for several indications. A nebulized formulation of this non-glycosylated r-α_1-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-α_1-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 α_1-PI is large scale production performed in transgenic dairy animals (t-α_1-PI): sheep [by PPL Therapeutics (UK) in partnership with Bayer Biologicals (USA), (Dalrymple & Garner, 1998;

Wright et al., 1991)], and goats [by Genzyme Transgenics Corporation (USA), (Ziomek, 1998)]. The transgenic α_1-PI recovered from sheep milk was purified to 99.9% purity. Even so, sheep native α_1-PI and sheep α_1-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 α_1-PI. None of the subjects had an antibody response to human t-α_1-PI (Tebbutt, 2000; Spencer et al., 2005); however, antibody responses were observed to sheep α_1-PI and to sheep α_1-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-α_1-PI for therapeutic use

The general regulatory requirements for biologicals intended for therapeutic use, including r-α_1-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-α_1-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 α_1-PI, from the human r-α_1-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-α_1-PI from the circulation (Casolaro et al., 1987; Cantin et al., 2002a). Aberrant glycosylation (or lack of glycans) does not neccessarily affect biological activity of the recombinant protein, but it is important for its stability. According to recently published data, glycosylation of α_1-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-α_1-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 α_1-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 α_1-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-α_1-PI.

3.3 α_1-PI –based therapies

3.3.1 Safety and efficacy of intravenous α_1-PI augmentation

The intravenous augmentation of α_1-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. α_1-PI augmentation is assumed to slow down the rate of emphysema development and progression and, thus, to improve the life quality and duration of α_1-PI deficient patients, yet the essential proof of efficacy is missing. According to Hubbard & Crystal (1990), only approximately 2-3% of infused α_1−PI actually reaches the lungs; and the effictiveness of α_1-PI replacement therapy has been evaluated mainly on the bases of biochemical (not clinical) criteria (Tonelli & Brantly, 2010). For recently approved α_1-PI products, their pharmacokinetic equivalence and comparable safety profile to Prolastin were demonstrated (*e.g.*, Stocks et al., 2010). α_1-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 α_1-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 α_1-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 α_1-PI products

Due to the inconvenience of life-time intravenous augmentation therapy and low levels of α_1−PI reaching lungs, the inhalation of aerosolized α_1-PI has been suggested as a less invasive and more efficient way to deliver large amounts of α_1-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 α_1-PI deficiency has been proposed two decades ago (Hubbard et al., 1989; Hubbard & Crystal, 1990), there is still no α_1-PI aerosolized treatment approved. Several studies examined efficiency of the α_1-PI inhalation therapy in animals and in humans (Kropp et al., 2001; Siekmeier, 2010). It was demonstrated (Kropp et al., 2001) that significantly more α_1-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 α_1-PI, it is generally assumed that products directly delivered to the lungs may not require the same degree of stability as α_1-PI given intravenously. However, as mentioned above, human studies using r-α_1-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 α_1-PI applications

Currently, α_1-PI therapeutic preparations are licensed exclusively for one indication, *i.e.*, chronic augmentation and maintenance therapy in individuals with emphysema due to congenital α_1-PI deficiency. Previously unrecognized inherited disorder, α_1-PI deficiency was first described in 1963 (Laurell & Eriksson, 1963) based on the serum electrophoretic analysis that revealed five individuals deficient of α_1–fraction; three of those patients had developed emphysematous conditions. Six years later, in 1969, cirrosis associated with α_1-PI deficiency was described (Sharp et al., 1969). These findings initiated a concept of linkage between α_1-PI deficiency and pulmonary and liver diseases. As evident from the available literature, due to the multiple biological activities of α_1-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 α_1-PI augmentation therapy (see recent reviewes by Blanco et al., 2011 and Janciauskiene et al., 2011).

According to Blanco et al. (2011), α_1-PI therapy has proven remarkable efficacy in small cohorts of α_1-PI-deficient patients who also suffer from fibromyalgia, systemic vasculitis, relapsing panniculitis and bronchial asthma. Although the putative benefits of α_1-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 α_1-PI treatment, in general it indicates a clear potential for additional α_1-PI supply to satisfy the anticipated clinical demand in near future. Because of controversy related to the additional clinical implications of α_1-PI deficiency, more clinical data are needed to verify whether the reported links between α_1-PI deficiency and other rare diseases are real or accidental.

As a potent anti-inflammatory agent, α_1-PI has been investigated in clinical studies for treatment of cystic fibrosis (Jones & Helm, 2009). Whereas patients with emphysematous conditions suffer from the hereditary α_1-PI deficiency and, thus, insufficient levels of the protease inhibitor in the lungs due to impaired α_1-PI synthesis in hepatocytes, patients with cystic fibrosis may have normal synthesis of α_1-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 α_1-PI augmentation therapy to prevent the deleterious effect of free protease (Allen, 1996; Birrer, 1995; Birrer et al, 1996) However, intravenous administration of α_1-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 α_1-PI in cystic fibrosis and α_1-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 α_1-PI both in patients with cystic fibrosis and in those with α_1-PI deficiency came to positive conclusions regarding deposition of inhaled α_1-PI in the lungs and its anti-elastase activity (see review by Siekmeier, 2010), the conclusion from other studies was that treatment with α_1-PI did not demonstrate any clinical improvements (Martin, 2006). If further clinical studies support the safety and efficacy of an aerosolized α_1-PI, and it is approved for treatment of cystic fibrosis, the demand for therapeutic α_1-PI preparations could be significantly increased.

Disease	References
Vasculitis	Dowd et al., 1995; Esnault, 1997; Griffith et al., 1996
Panniculitis	Chowdhury et al., 2002; Gross et al., 2009; Kjus et al., 2002; Smith et al., 1987; Valverde et al., 2008
Fibromyalgia	Ablin et al., 2009; Blanco et al., 2004; Blanco et al., 2010
Asthma	Blanco et al., 2008; Blanco et al., 2011; Eden et al., 1997
Pancreatitis	Rabassa et al., 1995; Needlham & Stockley, 2004
Renal	Szönyi et al., 2006; Ting et., 2008
Diabetes	Kalis et al., 2010; Lisowska-Myjak et al., 2006;
Cancer	Li et al., 2011; Lindor et al., 2010; Topic et al., 2011
Rheumatoid arthritis	Grimstein et al., 2010; Grimstein et al., 2011
Atherosclerosis	Stakisaitis et al., 2001; Talmud et al., 2003;
Acute anterior uveitis	Fearnley et al., 1988; Saari et al., 1986
Chronic rhinosinusitis	Kilty et al., 2008, 2010; Maune et al., 1995

Table 3. Conditions other than emphysema and liver disease possibly associated with α_1-PI

3.3.4 Research toward the enhancement of α_1-PI-therapies

During last decade various approaches have been considered for the enhancement of α_1-PI-based therapies. For instance, to prolong a short half-life of r-α_1-PI in the circulation, Cantin and co-workers hypothesized that conjugation of r-α_1-PI with polyethylene glycol (PEG) at Cys^{232} 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 Cys^{232} of nonglycosylated r-α_1-PI (human) results in an active inhibitor with extended *in vivo* stability. Moreover, 72 h later after airway instillation, the PEG-r-α_1-PI seemed to be significantly better than glycosylated α_1-PI at protecting the lung against elastase–induced lung hemorrhage.
As an example of the *in vitro* biochemical evaluation of the concept, α_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 α_1−PI therapies (Karnaukhova et al., 2010). For instance, α_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 α_1−PI augmentation therapy cannot cure, but may only slow down, the progression of emphysema, its complexation with retinoic acid could be more efficient for treatment than α_1−PI alone. It is noteworthy that the interactions of α_1−PI with several other physiologically active ligands (including porphyrins) may reveal additional properties of this multifunctional serpin.

4. Conclusions

Since α_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 α_1-PI structure-function relationships and clinical manifestations of α_1-PI deficiency has increased tremendously. Moreover, multi-disciplinary research efforts prompted the development of α_1-PI-based augmentation therapy to maintain the inhibitor level above the protective threshold. Since 1987, several α_1-PI products derived from pooled human plasma have been approved and are currently available to slow down the progression of emphysematous conditions in α_1-PI-deficient patients. In addition, due to its multiple physiological activities, α_1-PI has been identified for its putative involvement in several other rare diseases, the treatment of which may possibly benefit from α_1-PI-based therapies. As an alternative to intravenous administration that may improve the efficacy of α_1-PI treatment, the inhalation of aerosolized α_1-PI preparations has been in clinical trials. Recombinant versions of human α_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 α_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 α_1-PI products, it is essential that the efficacy of α_1-PI replacement therapy be clearly demonstrated in prospective, randomized, placebo-controlled trials. Will the efficacy of inhalation therapy using aerosolized α_1-PI preparations be proven to be superior to that of the intravenous route? Will the recombinant/transgenic versions of human α_1-PI be optimized to meet the requirements for protein therapeutics? Will other rare diseases currently implicated in association with α_1-PI and α_1-PI deficiency be clearly proven to benefit from α_1-PI treatment? From the standpoint of product quality, safety and efficacy, the current state of research and development of α_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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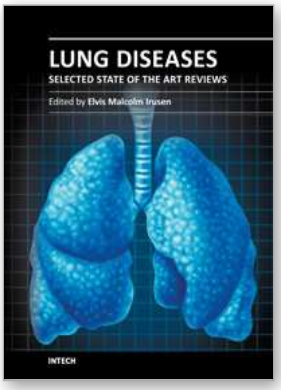

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