Review

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Update on alpha-1 antitrypsin deficiency: New therapies

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Summary

α_1-Antitrypsin deficiency is characterised by the misfolding and intracellular polymerisation of mutant α_1-antitrypsin within the endoplasmic reticulum of hepatocytes. The retention of mutant protein causes hepatic damage and cirrhosis whilst the lack of an important circulating protease inhibitor predisposes the individuals with severe α_1-antitrypsin deficiency to early onset emphysema. Our work over the past 25 years has led to new paradigms for the liver and lung disease associated with α_1-antitrypsin deficiency. We review here the molecular pathology of the cirrhosis and emphysema associated with α_1-antitrypsin deficiency and show how an understanding of this condition provided the paradigm for a wider group of disorders that we have termed the serpinopathies. The detailed understanding of the pathobiology of α_1-antitrypsin deficiency has identified important disease mechanisms to target. As a result, several novel parallel and complementary therapeutic approaches are in development with some now in clinical trials. We provide an overview of these new therapies for the liver and lung disease associated with α_1-antitrypsin deficiency.

Keywords: Serpins; Cirrhosis; Emphysema; Therapeutic strategies; Polymerisation.

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Introduction

We have described a group of protein conformational diseases that we have termed the serpinopathies [1]. They are characterised by the misfolding and intracellular polymerisation of members of the serine protease inhibitor or serpin superfamily. The best characterised of the serpinopathies is α_1-antitrypsin deficiency [2]. This is one of the most common genetic disorders with the severe Z deficiency allele (Glu342Lys) being present in 1:25 of the North European Caucasian population of whom 1:2000 are homozygotes. The Z mutation causes the retention of protein within hepatocytes in association with neonatal hepatitis, cirrhosis and hepatocellular carcinoma [3–5]. There is no specific treatment for the liver disease associated with α_1-antitrypsin deficiency, which accounts for 3.5% and 1.1% of paediatric and adult liver transplants in the UK respectively.

The lack of α_1-antitrypsin, an important protease inhibitor, predisposes the Z homozygote to early onset panlobular basal emphysema [6]. α_1-antitrypsin deficiency is the only known genetic cause of emphysema and is found in 1-2% of all individuals with chronic obstructive pulmonary disease (COPD); COPD will be the third commonest cause of death worldwide by 2020. The only treatment directly targeting the underlying pathobiology of the lung disease is α_1-antitrypsin augmentation therapy, which costs approximately 100,000 USD/patient/year. α_1-Antitrypsin deficiency accounts for 3.2% of adult lung transplants and 10% of all lung transplants for emphysema in the UK. Our work over the past 25 years has led to a new paradigm for α_1-antitrypsin deficiency. Here we review the molecular basis of α_1-antitrypsin deficiency and provide an update of the therapeutic strategies that are being developed.

Polymerisation: the central feature of α_1-antitrypsin deficiency

α_1-Antitrypsin is the archetypal member of the serpin superfamily. The wild-type M protein is a 394 residue, 52 kDa glycoprotein that is synthesised by hepatocytes, but is also produced by lung and gut epithelial cells, neutrophils and alveolar macrophages. α_1-Antitrypsin is the major circu-

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Key point

α_1-Antitrypsin deficiency is characterised by the intracellular polymerisation of mutant protein within the endoplasmic reticulum of hepatocytes.

lating antiprotease but its key function is regulation of the proteolytic effects of neutrophil elastase within the lung. We showed that the severe Z deficiency mutant of α_1-antitrypsin is retained within the endoplasmic reticulum (ER) of hepatocytes as ordered polymers that become sequestered in periodic acid Schiff-positive, diastase-resistant inclusions [2,7]. We have used biophysical and crystallographic techniques to dissect the pathway of α_1-antitrypsin polymerisation [8–12]. Our work suggests that the Z mutation perturbs its local environment (breach region, Fig. 1A) to favour population of an unstable intermediate that we termed M* [8] in which β-sheet A opens [2,8] and the upper part of helix F unwinds [9,13,14]. The patent β-sheet A can then accept insertion of the reactive site loop motif. Sequential insertion of the loop of one α_1-antitrypsin molecule into β-sheet A of a neighbour to form first a loop-sheet dimer, and then longer species linking more molecules, is the simplest model to explain the formation of elongated polymers [2,8,15] (Fig. 1B (i)). Indeed, polymerisation is blocked by peptides that mimic the reactive loop sequence and so compete for binding to the insertion site in β-sheet A [2,16]. We subsequently showed that the same process explains the profound plasma deficiency and hepatic inclusions of 3 other mutants of α_1-antitrypsin: Siiyama (Ser53Phe) [17], Mmalton (ΔPhe52) [18] and King's (His334Asp) [7]. Polymerisation also underlies the deficiency of the mild S (Glu264Val), I (Arg39Cys), Queen's (Lys154Asn) and Baghdad (Ala336Pro) alleles of α_1-antitrypsin [12,14,19,20] but the rate of polymer formation is much slower in keeping with the absence of liver disease and only mild plasma deficiency. In many cases the reduction in the thermal stability of the native fold of α_1-antitrypsin caused by mutations directly correlates with the polymerogenic tendency [11]. This implies that the mutants' conformational behaviour is qualitatively similar to that of wild-type α_1-antitrypsin, but that destabilised states become accessible at lower temperatures. However in some cases, disease mutations appear to cause polymerisation more by altering the balance of conformational behaviour (kinetic destabilisation) between native and intermediate states to favour population of the latter [21]. Importantly, one such exception is the Z variant that appears to cause a relatively mild thermal destabilisation but is highly polymerogenic [11,12,16]. Understanding the polymerogenic behaviour of the most clinically-significant variant is important as it has relevance for the readouts that may be used in screening for therapeutic agents [16,22]. Conversely, understanding the behaviour of rarer or milder variants in addition opens the way to precision medicine approaches, analogous to recent advances in cystic fibrosis [23,24].

Controversies on the structure of the pathological polymer

Our original description of polymers of Z α_1-antitrypsin envisaged a linkage between the reactive centre loop and β-sheet A [2] (Fig. 1B (i)). Clinically, Z α_1-antitrypsin inclusions within hepatocytes are increased by pyrexia and we assumed that polymers generated *in vitro* by heating purified Z α_1-antitrypsin would be identical to those formed *in vivo*. We further assumed that polymers generated *in vitro* would be identical whether they were formed by heating purified Z α_1-antitrypsin or incubation with denaturants (urea or guanidine) [8]. However we now know the latter assumption was incorrect, and the former assumption is also a matter of debate [22]. An alternative linkage was suggested by the crystal structures of a dimer of antithrombin in which the molecules were linked by a β-hairpin of the reactive centre loop and strand 5A [25] (Fig. 1B (ii)). The biophysical characteristics of polymers of α_1-antitrypsin formed by refolding from guanidine gave support to the β-hairpin linkage [26]. The cause of the disparate findings became clear with our development of the 2C1 monoclonal antibody that recognises polymers from the livers of individuals with α_1-antitrypsin deficiency [7]. This antibody binds polymers formed by heating monomeric α_1-antitrypsin, but not those formed by refolding from guanidine and urea [15]. Our nuclear magnetic resonance (NMR) studies followed the polymerisation of Queen's (Lys154Asn) α_1-antitrypsin under physiological conditions or in urea. Intermediate (M*) formation under physiological conditions was associated with highly native-like behaviour with changes in a few key motifs [14]. Global changes were observed in urea consistent with more widespread unfolding, in keeping with data from hydrogen-deuterium exchange [27]. Consequently different polymeric linkages can be accessed by different denaturing conditions. The application of heat to monomeric α_1-antitrypsin recapitulated the 2C1 neo-epitope of polymers associated with disease [15]. This neo-epitope is also observed in self-terminating trimers of Z α_1-antitrypsin artificially constrained by disulphide bonds and purified from the cytosol of a yeast expression system [28]. This species was crystallised and the structure determined to 3.9 Å resolution. The structure revealed yet another stable linkage mechanism, this time via complementary intermolecular insertion of the C-terminal triple-strand motif (Fig. 1B (iii)). This