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Review Article

Biomedical Significance of Alpha$_1$-Antitrypsin: An Overview

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Abstract

Alpha$_1$-antitrypsin (α_1-AT) is a natural, endogenous inhibitor of serine proteases with preferential activity towards neutrophil elastase (NE), the enzyme that possess the ability to cleave important connective tissue protein, elastin. The normal circulating concentrations of α_1-AT is thus very important to maintain the elasticity of tissues. Severely diminished levels of α_1-AT are associated with development of various disease conditions, in particular disorders of lung. This review highlights the biological role of α_1-AT and its involvement in diseases. New therapeutic approaches involving α_1-AT are also discussed.

Keywords: Alpha$_1$-antitrypsin, Endogenous inhibitor, Neutrophil elastase, Serine protease, Serpin.

Introduction

Proteases (also Proteinases, Peptidases) are hydrolases and are degradative enzymes which cleave proteins into smaller peptides and amino acids.[1] They represent a class of enzymes which occupy a central position with regard to their biological functions and commercial applications. Proteases are classified into six different types viz; serine proteases, aspartic proteases, cysteine proteases, threonine proteases, glutamate proteases and metalloproteases. There are at least 500 to 600 different proteases in humans; most of them are serine, cysteine, and metalloproteases.[2] The regulation of protease activity in tissues is a prerequisite for the maintenance of homeostasis. They may be potentially damaging when present at high concentrations. So they are tightly regulated in the extracellular and pericellular space to avoid degradation of connective tissue proteins such as elastin, collagen and proteoglycans.[3] The basic level of control is normally achieved by regulated expression/ secretion, by activation of inactive precursors or zymogens of proteases, and by degradation of the mature enzymes.[4] A second level of regulation is by inhibition of their proteolytic activity by endogenous protein inhibitors.[3-5]

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The proteinase inhibitory activity of human plasma was recognized in 1894 by Fermi and Pernossi.[6] The main inhibitor of proteolytic activity was first isolated in 1955 by Shultze and named it alpha$_1$-antitrypsin (α_1-AT) because of its ability to inhibit trypsin.[7] It is now recognized that α_1-AT is not only a potent inhibitor of trypsin but also inhibits several serine proteases with a preferential activity towards neutrophil serine proteases, neutrophil elastase (NE) and proteinase-3.[8] and is one of the most abundant serine protease inhibitors (serpins) in the circulation.[7-9]

α_1-AT is a polymorphic, acute-phase glycoprotein synthesized mainly in hepatocytes(70-80%) and secreted into the plasma. Besides liver, it is also synthesized by extrahepatic tissues and cells, such as neutrophils, monocytes and macrophages, alveolar macrophages, intestinal epithelial cells, carcinoma cells and the cornea.[10-15] The normal plasma concentration of α_1-AT ranges from 0.9 to 1.75mg/ml and has a half-life of 3-5days. The

presence of α_1-AT is well established in other body fluids such as saliva, tears, milk, semen, urine and bile.[16-20]

Molecular Architecture

α_1-AT is a single-chain, globular glycoprotein consisting of 394 amino acids and a total molecular weight of 52kDa in its mature form. The main characteristics of the protein are: Met358 residue at the active site, isoelectric points ranging from 4.4 to 4.6. Crystallographic analysis of the mature protein reveals that it exhibits a number of glycoforms. There are three N-linked glycosylation sites on the external surface of the one end of the molecule. The side chains are composed of N-acetyl glucosamine, mannose, galactose and sialic acid and they are linked to amino acids Asn46, Asn83 and Asn247.[3,12] The internal structure of α_1-AT is highly ordered with 30% α-helices and 40% β-pleated sheets. α_1-AT is comparable to the general structure of Serpins having nine α-helices and three β-sheets with an exposed reactive center loop (Fig 1).

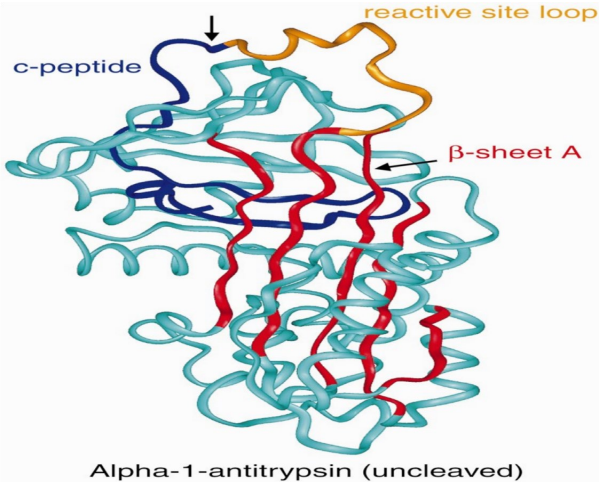

Fig 1. Schematic picture of the native α $_1$-antitrypsin. The reactive center (yellow), β-sheet A (red) and C-terminal peptide (dark blue) are shown in relation to the rest of the structure (light blue).

α_1-AT inhibits proteinases through the formation of a 1:1 molar α_1-AT-enzyme complex. It is a "suicide" or "single use" irreversible inhibitor that employs a unique and extensive conformational change in the process of inhibition of target proteases.[21]

Genetic Aspects

α_1-AT is encoded by SERPINA1 gene located in proteinase inhibitor (Pi) locus on the long arm of chromosome 14 (14q32.1). SERPINA1 is a highly polymorphic gene, with more than 125 single nucleotide polymorphisms (SNPs) reported in public SNP databases. More than 100 different naturally occurring genetic variants of α_1-AT have been identified. These normal variants of α_1-AT have normal serum level and functional activity to inhibit NE. More than 95% of normal variants are the "common" M1 (Ala213), M1 (Val213), M2 and M3.[3,12,22]

Several mutations associated with α_1-AT deficiency (α_1-ATD) have been identified, and the most common are Z and S types. The classical form of α_1-ATD is characterized by a point mutation (Glu342→Lys) that leads to misfolding of mutant α_1-ATZ. α_1-ATZ accounts for almost 95% of all cases of α_1-ATD.[3,12]

Biological functions

Alpha$_1$-Antitrypsin exhibits diverse roles in all living cells. One of its primary physiological roles is to inhibit the proteolytic activity of elastase secreted from activated neutrophils, thereby maintain the structural integrity of elastic tissues.[7,23,24] A balance between elastase and α_1-AT is essential to maintain normal integrity of tissues and an imbalance in favor of elastase has been implicated in the pathogenesis of several acute and chronic inflammatory diseases. Leucocytes, neutrophils and macrophages, which secrete large quantities of oxidants at sites of inflammation, were shown to induce oxidative inactivation of α_1-AT in vivo and this result in disturbed protease -antiprotease balance.[25]

Until recently it was thought that inhibition of NE is the primary function of α_1-AT. However, current studies have demonstrated that α_1-AT expresses anti-inflammatory,[8] antiapoptotic,[26-29] Immunomodulatory,[30,31] and antimicrobial effects.[32-34] α_1-AT is an irreversible inhibitor for kallikriens 7 and 14.[35] Anti-inflammatory action of α_1-AT is facilitated by blockade of pro-inflammatory cytokine release from human peripheral blood mononu-

clear cells. Specifically, α_1-AT decreases the production of two typical upstream mediators of inflammation TNF-α and IL-1β. α_1-AT is also shown to increase the levels of IL-10, an anti-inflammatory cytokine in various experimental conditions.[36] The activity of pro-inflammatory cytokines appeared to consistently diminish in the presence of elevated α_1-AT, with an increased release of anti-inflammatory mediators.[37] α_1-AT also lowers the levels of the IL-8 and monocyte chemotactic protein (MCP)-1, two major chemokines involved in the chemotaxis of inflammatory cells. α_1-AT has also been reported to inhibit neutrophil superoxide production.[38]

Recent studies have demonstrated that α_1-AT inhibits the activity of caspase-3, an intra-cellular cysteine protease which plays an essential role in cell apoptosis.[28,29] Studies have shown α_1-AT to have stimulatory effect on insulin secretion and protects β-cells against cytokine-induced apoptosis. Animal experimental studies provide further evidence that α_1-AT therapy prolongs islet graft survival in transplanted allogenic diabetic mice. Invitro studies have demonstrated that over-expression of α_1-AT significantly reduces insulitis and prevents the development of overt hyperglycemia in non-obese mice.[39,40]

Antimicrobial roles of α_1-AT against *Escherischia coli* proteins.[32] *Cryptosporidum parvum*, a protozoan parasite.[33] and *Pseudomonas aeruginosa*.[34] has been established. It has recently been demonstrated that a specific 20-residue fragment of α_1-AT binds to the gp41 fusion peptide of HIV-1 and prevents the virus from entering target cells, thereby inhibiting HIV-1 infection. These findings suggest that α_1-AT may play a protective role in HIV-1-infected individuals.[41] Findings have also revealed a role of α_1-AT in iron metabolism that it enhances the synthesis of both transferrin receptor and ferritin.[42]

In Diseases and Therapy

Alterations in the structure and/or secretion of α_1-AT could lead to its decreased concentration or deficiency which is known to predispose the individual to diseases. Clinical expressions or manifestations of α_1-ATD can be seen in the lung, liver and skin.[43,44] In fact, α_1-ATD is the only known genetic risk factor for the development of chronic obstructive pulmonary disease (COPD), a chronic inflammatory disease of lung characterized by excessive proteolytic destruction of lung.[45-49] Experimental data show that cigarette smoke accelerates the polymerization of Z α_1-AT by oxidative modification, which further reduces pulmonary defenses and increases influx of neutrophils into the lungs leading to an elastase/antielastase imbalance that accelerates lung tissue breakdown.[50] These novel findings provide a molecular explanation for the development of premature emphysema in ZZ α_1-ATD subjects who smoke.[51] This observation has raised the prospect of anti-elastase therapy in α_1-ATD related COPD. Intravenous or intratracheal aerosol administration of recombinant or purified, plasma derived α_1-AT is now a standard therapy used for individuals with AATD and pulmonary emphysema.[52]

Z mutation of α_1-AT slows the folding of the molecule with subsequent increase in the concentration of intermediate which then polymerizes and accumulates in the endoplasmic reticulum of hepatocytes with reduced secretion resulting in lower or undetectable serum level of α_1-AT than normal variants.[53] This accumulation of abnormally folded α_1-AT protein leads to toxic consequences, including hepatic fibrosis/cirrhosis and carcinogenesis.[54,55] Retrospective studies have shown that up to 25% of those with α_1-ATD suffer from liver cirrhosis or liver cancer in late adulthood.[56]

A number of reports have also associated α_1-ATD with pancreatitis,[57-59] inflammatory bowel disease,[60,61] peptic ulcer,[62,63] coronary atherosclerosis,[64] renal disease,[65,67] panniculitis,[68,69] cancer,[70-72] etc. Few case reports provide evidence for the infusion of α_1-AT induces a rapid clinical resolution of panniculitis supporting α_1-ATD as a contributor to the pathogenesis of panniculitis.[73-75]

Recent studies have raised the possibility of an association between α_1-AT and diabetes. Clinical studies have demonstrated that

plasma α_1-AT levels and activity were lower in diabetic patients than in non-diabetic controls.[9,76] In a study on the levels of α_1-AT conducted by the authors at R.L.Jalappa Hospital and Research Centre, the teaching hospital of Sri Devaraj Urs Medical College, Kolar, in patients suffering from Type 2 diabetes mellitus, it was observed that there was a generalized and significant decrease in the levels of α_1-AT in diabetic patients compared with healthy controls. This generalized reduction could be probably due to the destruction of α_1-AT by reactive oxygen species (ROS), as α_1-AT is known to be destroyed by ROS.[77]

The study when extended to patients suffering from complications of prolonged hyperglycemia, we have observed a further decrease in the levels of α_1-AT in diabetic retinopathy (DR) and nephropathy (DN) complications. A comparison on the levels of α_1-AT between DR and DN yielded an important and vital observation that there was a significant reduction in the levels of α_1-AT in patients with DR in comparison to DN. The explanation for such an observation could be provided through the following possibilities viz: defective secretion of α_1-AT, decreased expression or mutation in the α_1-AT gene resulting in decreased synthesis or defective/truncated α_1-AT formation in diabetes retinopathy patients. The results of this study are promising and implicate that α_1-AT could have a predictive role in indicating the possibility of developing diabetic retinopathy (unpublished data).

In support of this, various research groups working on experimental diabetes in animal models and retinal pericyte cell cultures have demonstrated retinopathic type of changes in retinal pericyte cells and proposed potential, protective role of α_1-AT on retinal vasculature. It was also suggested that early use of α_1-AT therapy may be an effective strategy to prevent or hinder the progression of diabetic retinopathy. α_1-AT is currently under evaluation for treatment of Type 1 diabetes patients in multiple clinical trials. Initial results suggest that α_1-AT therapy could potentially improve insulin production without adverse effects.[78-80]

α_1-ATD is associated with several pregnancy and placental disorders. Studies have reported significantly lowered α_1-AT levels in severe preeclampsia women as compared to normal pregnant women.[81] An association between decreased levels of α_1-AT with the severity of preeclampsia was observed by us suggesting that it would be a relevant marker for assessment of severity of preeclampsia (unpublished data, Manuscript submitted to Journal of Clinical and Diagnostic Research). Feng et al in a proteomic study using 2D PAGE identified that the normal full-term pregnant women expressed the most α_1-AT and in late-onset PE women α_1-AT was down regulated. This differential expression was also consistent with the peripheral circulating levels of α_1-AT as the concentration was highest in full-term pregnancy group, moderate in the early-onset and lowest in the late-onset PE group.[82] A recent study by the same authors on preeclampsia animal model demonstrated that α_1-AT injection significantly relieved the high blood pressure, increased fetal weight and reduced urine protein levels in a dose-dependent manner and thus improve preeclampsia. Thus α_1-AT could be a potential strategy for preeclampsia therapy.[83]

α_1-AT with potent anti-inflammatory, antiapoptotic and cytoprotective along with its anti-elastase properties is tested by various researchers for its beneficial role in stroke. Konrad C et al reported a case report of a 45 year old male patient with homozygous α_1-ATD in whom spontaneous internal carotid artery dissection occurred in the absence of any other known risk factors.[84] In a study by Moldthan HL et al, intravenous/ intracranial delivery of human α_1-AT into rats with induced middle artery occlusion significantly reduced the infarct volume at 72 hours compared with control rats, concluding that human α_1-AT could be a potential novel therapeutic drug for the protection against neurodegeneration following ischemic stroke.[85] Reduction in the levels of α_1-AT in dengue viral infection was observed by us (unpublished data, Manuscript submitted to Indian Journal

of Clinical Biochemistry). Boutten A et al estimated α_1-AT concentrations in BAL fluid from both pneumonia-infected and non-involved lung and found elastase-inhibitory capacity of α_1-AT in the involved lung was reduced.[86]

Conclusion

In view of the profound and cardinal roles that α_1-AT plays under both physiological and pathological conditions, further research in this area may provide useful insights that will contribute to our understanding on the role of this multifunctional molecule in the pathogenesis of diseases and suggest new therapeutic strategies.

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