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EDITORIAL

The potential role of elastase inhibitors in emphysema treatment

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Several lines of evidence, albeit most of them circumstantial, support the protease-antiprotease imbalance concept in the pathogenesis of emphysema. Neutrophil elastase (NE) has become the key enzyme for several reasons. NE is insufficiently inhibited by α_1-antitrypsin (AAT) (α_1-proteinase inhibitor) in inherited deficiency states when the extracellular level of the predominant antiprotease AAT is low as in AAT-deficiency, PiZ, and other rare genetic variants. NE is the target enzyme of AAT, the rate of association between them being extremely rapid [1].

AAT is the archetype of the so called serpin (serine proteinase inhibitors) super family of highly homologous proteins, including the protease inhibitors α_1-antichymotrypsin (target: cathepsin G), antithrombin III (target: thrombin) and the protein C inhibitor. The serpins share the same overall tertiary structure [2]. The specific inhibitory activity of a serpin is determined by its reactive centre (the P_1-P_2 residues), a very short segment of the whole molecule. In AAT this centre is serine-methionine. The latter amino acid is easily oxidized by *e.g.* cigarette smoke components, leading to a partial inactivation of the elastase-inhibitory activity. This mechanism is thought to induce an acquired AAT-deficiency state, which may partly explain the high incidence of the garden variety of centrilobular emphysema seen in cigarette smokers [3]. The early onset emphysema in hereditary AAT-deficiency is typically basal and panlobular.

Domains outside the small reactive centre in the serpin molecule may have other functions, *e.g.* the C-terminal fragment of AAT is apparently effective in chemotaxis [4]. Similarly, extracellular NE may have multiple biological functions. Elastases break down elastin, a process called elastolysis, but are also capable of degrading a variety of other matrix components such as proteoglycans. The major role of intracellular NE is to degrade proteins ingested by leucocytes during phagocytosis. Other serine proteinases localized to the α-granulae of leucocytes are less important in elastolysis but are to some extent also inhibited by AAT, and have bactericidal properties such as cathepsin G and the recently isolated proteinase -3 [5].

Intratracheal administration of elastases (human or pancreatic) in animal models leads to emphysema with destruction of elastic fibres and loss of recoil pressure, the physiological hallmark of emphysema. The analogy with human emphysema is limited by the acute nature of the experimental procedure and the nonphysiological way in which the enzyme is administered. However, the experimental emphysema models are indispensable tools in the primary evaluation of antiproteolytic substances, which may have a potential application in human medicine.

Against this background, it is not surprising that investigators are increasingly engaged in the search for potent elastase inhibitors with a putative role of retarding the emphysematous process and inhibiting excessive proteolysis in many other clinical settings where the protease-antiprotease balance is disturbed. Powerful techniques, such as nuclear magnetic resonance (NMR) and site-specific mutagenesis, have facilitated studies on the structural-functional relationship of serine proteinases and their interaction with natural or modified serpins. A wide variety of small molecular synthetic inhibitors have appeared [6].

Chloromethylketone derivates are irreversible inhibitors, which can prevent emphysema development in hamsters if given before the elastase but are ineffective if given after elastase. These compounds are small molecules which can easily pass into the interstitium and exert their action. They are, however, unable to inhibit elastase that has already been bound to interstitial elastin. This problem is relevant when discussing elastase inhibitors, in view of the observation that NE accumulates on interstitial elastin in emphysema [7]. Although low molecular weight inhibitors may have a favourable pharmacokinetic profile, being well absorbed from the gut after per oral administration and rapid penetration into the interstitium, it seems important that they are not freely diffusable into cells in general and neutrophils in particular. This could have unwanted effects on the normal defence mechanisms exhibited by granulocytes.

Peptidyl boronic acids constitute another group of synthetic inhibitors, which are tight binding, reversible inhibitors of NE. They are relatively non-toxic, have a sufficient *in vivo* stability, and prevent elastase induced emphysema in hamsters. However, no human studies have been published.

Certain neutral cephalosporins represent a third group of potent irreversible inhibitors of mammalian serine proteinases. When elastase is released by exocytosis from

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stimulated neutrophils, inhibitors such as L-658, 758 exert their inhibiting effect extracellularly in a supplementary manner to that of AAT [8]. This low molecular weight synthetic inhibitor may well have a role in a variety of elastase-mediated diseases.

Alternatively, biosynthetically derived natural inhibitors may be used. These inhibitors may be of peptide or carbohydrate nature. It has been demonstrated that several anionic glycosaminoglycans (GAG), including heparin, which is a complex and heterogeneous mixture of carbohydrates, are capable of inhibiting NE *via* electrostatic interactions [9]. NE is a strong basic protein with a pI near 11 and heparin is strongly anionic due to its sulphation. In an interesting report in the previous issue of the Journal, LAFUMA *et al.* [10] demonstrated the ability of a low molecular (mean weight 4,500) heparin fragment (Cy 222) to prevent human elastase (NE) induced emphysema in hamsters. The fragment is prepared by extensive nitrous acid depolymerization of heparin. *In vitro* it was demonstrated that the inhibitory activity was decreased thirty fold by binding to the substrate elastin, but still the retained inhibitory capacity was high. The authors also made the important observation that the fragment could inhibit NE both in its free and elastin-bound state. Cy222 decreased the mean linear intercept by 70% after 8 weeks in the surviving (only 40%) hamsters. Heparin derivates may represent a new class of potential physiological inhibitors in prevention of emphysema. Other glycosaminoglycans may have potential value [11]. Predictable difficulties in human applications are route of administration and putative anticoagulative properties. The fragment is a good candidate for aerosol treatment considering its relatively low molecular weight. Not much is known, however, about the specificity of action. It seems very important that any inhibitor administered to man is highly selective in action, *i.e.* binds specifically to NE and does not interfere with other serine proteases. Glycosaminoglycans, including heparin, seem to accelerate the inhibition of serine proteases by serpins in general. One example based on the construction of a three-dimensional model of the serpin protein C-inhibitor (PCI) indicated a dominant role of electrostatic interactions in explaining the heparin-PCI binding [12].

Protein inhibitors share with GAGs the inability for per oral administration. The feasibility of *i.v.* administration of purified human AAT to patients with AAT-deficiency has been demonstrated [13]. Although cost benefit analyses are in favour of replacement therapy [14], so far no documentation of its effect in a controlled trial has appeared. The alternative of aerosol administration is interesting, but the size of the molecule (54 kD) probably prevents an effective penetration into the alveolar interstitium. Recombinant AAT has full biological activity despite being non-glycosylated and may be a better choice for aerosol treatment. A genetically engineered mutant of the AAT-gene contains a valine residue at position 358 in place of methionine. This mutant has antielastase activity but is resistant to oxidation. Administration of a non-oxidizable inhibitor may have undesired, dangerous effects; the halo of oxidizing agents (respiratory burst and myeloperoxidase) around the migrating neutrophil may be compromised and infectious defence mechanisms seriously damaged [15]. The use of human inhibitors or their derivates has many advantages, one being the minimal risk of immunization; the highly selective, stable and low molecular (8 kD) protein inhibitor eglin C illustrates this aspect. This potent inhibitor, which is isolated from the leech prevented NE-induced animal emphysema but had to be abandoned because of its potential to cause hypersensitivity reactions [16].

Perhaps the most fascinating human proteinase inhibitor with a potential as elastase inhibitor is the secretory leucocyte proteinase inhibitor (SLPI), a relatively small (11.5 kD), non-glycosylated peptide. In the lung it is produced in the secretory glands of the proximal airways, where it is the major antielastase. It consists of two homologous domains. Serine proteases bind only to the second domain in a reversible manner. The recombinant form of SLPI is similar in its *in vitro* properties to naturally occurring SLPI and is capable of protecting hamster lung parenchyma from NE-induced damage [17]. Furthermore, it has recently been shown that it is able to inhibit NE when bound to elastin. It has been suggested that SLPI is a unique inhibitor of contact-dependent proteolysis, the activity of which cannot be mimicked by other plasma proteinase inhibitors [18]. However, at present, not much is known about its pharmacokinetic properties.

Clearly numerous potent elastase inhibitors will be available to pneumologists, but many problems have to be solved before proceeding to large scale human studies. Regardless of which inhibitors, natural or synthetic, are finally tested in such studies, it is necessary to have access to improved methods to monitor emphysema. The difficulties in following the progress of emphysema by ordinary forced expiratory volume in one second (FEV_1) measurements has been extensively discussed in recent years [19]. New methods to follow the rate of the elastolytic process by biochemical methods are needed, and fortunately progress in this area of research now seems to be rapid. The radioimmunochemical assay of elastin-derived peptides is one promising approach [20]. The human elastin gene has been cloned, facilitating the synthesis of selected polypeptide domains of the molecule and the preparation of specific antibodies to those domains. An alternative approach is the assay of elastase-specific fibrinogen fragments. The NE-derived fibrinopeptide Aα 1-21 can be directly measured as well as its carboxypeptidase derived degradation products. A correlation has been found between circulating peptide levels and proteinase inhibitor phenotype. Higher peptide levels are found in smokers than in nonsmokers. These data support the use of fibrinopeptides as an index of NE activity [21]. Differences in assay techniques can probably explain the discrepant results of other groups [22]. Finally, measurement of urinary desmosine, an amino acid unique to elastin, is presently studied with improved methods [23] and evaluated as a marker of pulmonary elastolysis.

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