



LEUKOCYTE ELASTASE AND ANTI- ELASTASES IN PULMONARY EMPHYSEMA

A thesis submitted by

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ABSTRACT

Although the cause of emphysema is uncertain, the preferred theory to explain the aetiology points to an imbalance in the protease-antiprotease systems within the lung with human leukocyte elastase (HLE) and α_1 -protease inhibitor (α_1 -PI) being the main candidates. However an inherited deficiency of α_1 -PI, is responsible for only a small percentage of all cases of emphysema with the most frequent form occurring in some smokers who have normal α_1 -PI levels. This thesis examines some aspects of the protease-antiprotease theory.

The aim of the first part of this thesis was to examine the proposal that there may be a reduced capacity to inhibit HLE activity in serum and bronchoalveolar (BAL) fluid from emphysema patients and susceptible normal smokers when compared to normal individuals. Comparison groups included children, adult non-smokers, and patients with an acute lung disease, adult respiratory distress syndrome (ARDS). The serum α_1 -PI concentration was significantly increased in children and in adults with emphysema and ARDS. However, no reduction in the HLE inhibitory and the α_1 -PI functional capacity of serum samples from the emphysema and smoking groups was found when compared to the other groups studied, except in the ARDS group, where both were significantly reduced. BAL samples from volunteers were obtained from normal adult non-smokers and smokers and from patients with ARDS. The α_1 -PI concentration in BAL samples from ARDS patients was increased by more than 40 fold but was only 37% functional compared to the 70% and 85% seen in non-smokers and smokers respectively. However no significant reduction in the HLE inhibitory and the α_1 -PI functional capacity of BAL samples from smokers was noted when compared to non-smokers. The presence of other HLE inhibitors in addition to α_1 -PI was demonstrated by increased inhibition towards HLE in all BAL samples. In conclusion, no evidence was found for a reduction in the ability of serum and BAL fluid from emphysema patients and normal smokers to inhibit HLE activity that could explain the increased risk of development of emphysema in smokers.

The second part of this study was to assess the effectiveness of a number of nonspecific and specific inhibitors towards HLE under varying assay conditions including the presence of lung and other surfactants. Overall heparin and the specific HLE inhibitor, ICI 200355, were found to be the most potent inhibitors of HLE using MeO-Suc-A-A-P-V-NA as substrate, although heparin is only most effective as a partial inhibitor (approx. to 25% of residual HLE activity). However, nonspecific proteins such as elastin peptides and even albumin, if present at a high enough concentration (50% inhibition seen with 6 g/l), were found to be capable of partial inhibition of HLE. The effectiveness of glycosaminoglycan (GAG) inhibition of HLE was, under certain conditions, found to be dependent on factors, such as the type of GAG, the HLE assay substrate, the degree of GAG sulphation and the size of the GAG. For example, heparin was the more effective inhibitor with the soluble substrate Suc-A-A-A-NA while heparan sulphate was more effective with insoluble lung elastin as substrate. In general, less inhibition was found with GAGs that were less sulphated and with low molecular weight GAGs (<2000). Cathepsin G, is not completely inhibited by the major protease inhibitors, α_1 -PI or α_2 -M, unlike HLE, the other major inflammatory serine protease. Pulmonary surfactant had a mild inhibitory effect on HLE activity whereas non-ionic surfactants, such as Brij 35, were found to not only activate HLE but also reduce the effectiveness of some inhibitors, particularly α_2 -macroglobulin and those GAGs which are poor inhibitors of HLE.

In the third part of this study high affinity binding fractions towards HLE were isolated from heparin to determine if heparin has some specificity of binding towards HLE. Sulphate content of the heparin fractions fell with increased binding and inhibition of HLE, which suggests that maximum inhibition and binding depend on an ordered sulphate group sequence rather than simply having the strongest polyanion. Heparinase digestion of heparin bound to HLE allowed the isolation of the most tightly bound heparin saccharide fractions that are likely to contain the high affinity binding sequences for HLE.

In conclusion, the significant findings from this study has shown that there was no reduction in HLE and α_1 -PI functional capacity in adults who smoked or who had emphysema. There was also a significant contribution of other inhibitors in addition to α_1 -PI in all BAL samples examined. In addition to α_1 -PI, a number of other compounds were shown to inhibit HLE, including heparin and heparan sulphate, which is clinically significant because of their strong inhibition of HLE and the large amounts of heparan sulphate proteoglycans and heparin that are found in the lung. This study also highlights the importance of the modifying effects of surfactants, including pulmonary surfactant, towards HLE activity and the function of various inhibitors towards HLE. Finally, a study of the interaction of heparin with HLE has revealed new information on the type and degree of specificity of the binding of heparin for HLE.

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