The Role of Free Radicals and Neutrophil Elastase in Development of Pulmonary Emphysema
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Extracellular proteolysis is hypothesized to be the major cause of pulmonary emphysema and oxygen-derived free radicals and neutrophil elastase are thought to play an important role in its pathogenesis. In this study, peripheral polymorphonuclear leukocytes (PMNs) obtained from 16 patients with emphysema generated a significantly larger amount of superoxide and elastase activity than those obtained from normal controls. A significant correlation was observed between elastase activity and superoxide release. In addition, the superoxide release showed a negative correlation with the disease duration. The superoxide release appeared to correlate with a decline of FEV₁ over the course of several years in 8 patients. It seems likely that activated PMNs play an important role in the development of pulmonary emphysema.

Key words: superoxide anion, polymorphonuclear leukocytes (PMNs)

Introduction

Pulmonary emphysema is characterized by the destruction of the alveolar wall with enlargement of the distal airspaces. It is widely held that an increase in proteolytic activity in the interstitium of the lung is the major cause (1, 2). Normally neutrophil elastase, a powerful protease capable of cleaving the structural backbone of the alveolar walls, is regulated by α₁-antitrypsin (α₁-AT) which is its major inhibitor (3, 4). A deficiency in α₁-AT has been linked to pulmonary emphysema (5). In addition, recent studies indicate that inflammatory cells down-regulate the activity of α₁-AT such that progressive destruction of the lung parenchyma may occur in some individuals and lead to emphysema (6, 7). Superoxide anion, hydrogen peroxide, hydroxyl radical and hypohalide anion produced by stimulated inflammatory cells (8, 9) all decrease the inhibitory capacity of elastase by oxidizing the methionine residues of α₁-AT (10). In vitro studies have shown that the macrophages in the alveoli of cigarette smokers spontaneously release such oxidants as superoxide anion and hydrogen peroxide (11). In addition, cigarette smoking is associated with a shift in the type of cells in the alveoli, such that the neutrophils, usually not present become represented in large numbers (12). Although the elastase antielastase theory of emphysema has gained wide acceptance, the pathogenesis of emphysema appears to be more complex than this theory can accommodate. The purpose of this study is to examine whether or not an imbalance exists which may enhance extracellular proteolysis in emphysema patients. Specifically, we examined the possibility that a potentiation of both the free radicals and neutrophil elastase from PMNs may be linked to the tissue damage associated with pulmonary emphysema and its progression.

Method

Subjects

Sixteen patients with established pulmonary emphysema participated in this study [mean age 62.5 (range 54–72)]. Diagnosis was based on clinical history, pulmonary function tests, and radiologic findings (Table 1). All patients had a history of smoking but had not smoked for several years. None had signs suggestive of respiratory infection at the time of the study, and none were receiving oral corticosteroid. As a control we studied nine healthy volunteers [mean age 60.8 (range 45–78)]. None had a history of chronic disease.

Isolation of Peripheral PMNs

Human neutrophils were isolated from heparinized venous blood through the sequential application of
Table 1. Characteristics of the Study Population

1) Age 62.5 ± 4.8

2) Gender (M/F) 13/3

3) Pulmonary function tests
   % VC (%) 74.7 ± 22.1
   FEV1 (% 1.25 ± 0.48
   % FEV1,0 (%) 50.3 ± 16.6
   RV/TLC (%) 54.8 ± 9.9
   % DLCO (%) 61.0 ± 25.0

Ficoll-Hypaque centrifugation (13) and 3% dextran sedimentation. Contaminating erythrocytes were lysed by exposure to hypotonic saline. The isolated neutrophils were washed and resuspended in Hank’s balanced salt solution (HBSS) at a concentration of 10^6 cells/ml. This procedure yielded cells with a viability of at least less than 95% by trypan blue dye exclusion and purity of at least 98% by May-Grunwald.

Superoxide anion (O_2^-) and neutrophil elastase assay

O_2^- generation was assessed by the superoxide dismutase (SOD) inhibitable reduction of ferricytochrome C (14). Neutrophils (1 x 10^6/ml) were suspended in HBSS and incubated at 37°C for 10 minutes. Cytochrome C and either PMA (phorbol myristate acetate, 10 ng/ml final concentration) or fMLP (n-formyl methionyl leucyl phenylalanine, 10^-7 mol/l final concentration) were added to the cell suspension. PMA and fMLP were dissolved in dimethylsulfoxide (DMSO). Addition of DMSO alone was ineffective. The reaction was terminated after 10 minutes by cooling the sample to 0°C and centrifuging it at 2,000 rpm for 10 minutes. The supernatant was then decanted and the amount of reduced cytochrome C produced was assessed by reading at 550 nm in spectrophotometer. All reactants plus SOD were used in the control experiment. The amount of O_2^- produced was calculated based upon the amount of cytochrome C reduced in each tube, using an extinction coefficient of 2.95 x 10^4 M^-1 cm^-1 (15). Neutrophil elastase was assayed by following the hydrolysis of a specific substrate, [succinyl-L trianyl-p-nito anilide (SAPNA)] as described previously (16). Briefly, samples of 0.1 ml of cell suspension (1 x 10^6/ml) were added to 0.9 ml of buffer (0.05 M Tris at pH 7.5, with 0.5 M NaCl, 0.1 M CaCl_2) containing 0.5 mg/ml of the SAPNA substrate. The mixture was incubated at 37°C for 60 minutes and the supernatants were recorded the absorbance at 410 nm to measure release of nitroaniline. A standard curve was constructed employing porcine pancreatic elastase (PPE).

Statistical method

Data obtained were expressed as the mean ±SD and were analyzed using Student's t-test. Significance was determined at the p < 0.05 level.

Results

PMNs obtained from all subjects were found to generate superoxide anion and neutrophil elastase activity in vitro. However, there were significant difference (p < 0.01) in the release of superoxide anion in the presence of fMLP or PMA between the emphysema group (fMLP: 1.46 ± 0.51 nmol/min, PMA: 2.58 ± 0.71 nmol/min) and the control group (fMLP: 0.8 ± 0.2 nmol/min, PMA: 1.50 ± 0.4 nmol/min) (Fig. 1). Similarly, significant differences (p < 0.01) were seen in neutrophil elastase activity between the emphysema group (0.13 ± 0.03 µg PPE/ml) and the control group (Fig. 2).
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(0.06 ± 0.03 μg/ppE/ml) (Fig. 2). A significant correlation was observed between the elastase activity and superoxide release (Fig. 3). Thus, the peripheral PMNs from the emphysema patients were activated and had the capacity to generate a large amount of superoxide and elastase activity. In addition, the relationship between the superoxide release and the time of onset of exertional dyspnea was evaluated according to the patient's history. The superoxide release shows a negative correlation with the time of symptom onset (Fig. 4). Also the decline in pulmonary function was calculated for eight emphysema patients for whom successive spirometric studies of FEV₁ (ΔFEV₁/year) were available. The superoxide release was correlated with the degree of annual decrease of FEV₁.0 (Fig. 5).

Discussion

In this study, we observed that PMNs from patients with emphysema were either activated or primed by various factors and generated a significantly larger amount of oxygen free radicals and neutrophil elastase as compared to normal controls. Both factors potentiate the process of extracellular proteolysis in the lung. Selby et al reported that neutrophil retention in the lung is higher in emphysema patients (17). Therefore these neutrophils resulted in an increased elastase burden in the lung. Accordingly, the activated neutrophils in our in vitro study might contribute to the development of pulmonary emphysema. A progressive decrease in pulmonary function ultimately leads to chronic respiratory insufficiency. PMNs from emphysema patients may release a massive amount of oxygen free radicals, which would then inactivate α₁-AT and enhance the elastase induced proteolysis. Further, the activated PMNs would secrete more neutrophil elastase and potentiate the development of pulmonary emphysema. Patients with emphysema might experience a disturbance of both their protective and aggressive factors and thus suffer from a proteolysis cascade in the lung parenchyma. In this study, the activity of superoxide released from the PMNs of emphysema patients showed a significant correlation with the annual decrease of FEV₁. Although the number of patients is too small to derive a firm conclusion, it can be speculated that the progression of pulmonary functional derangement might be predicted from the measurement of superoxide released from peripheral PMNs in vitro. We determined the reproducibility of superoxide released in these subjects. Although cigarette smoking appears to be the single most important causal factor in the development of pulmonary emphysema, only 10 to 15 percent of smokers develop clinically significant emphysema (18). Thus we have yet to identify the other factors that predispose to its development. Cigarette smoke can inactivate α₁-AT and would thus enhance the biological effects of secreted elastase. Neutrophils migrate through the interstitium of the lung and lead to the extracellular digestion of connective tissue. Burnet et al (19), found an enhanced chemotactic response to fMLP in neutrophils from emphysema patients. In fact, cigarette smokers show an increase in neutrophil elastase activity in the peripheral blood and broncho-alveolar lavage fluids (20). Mac Nee et al (21), have concluded that cigarette smoke in the lung increases the local concentration of neutrophils and that emphysematous lesions are the result of
the destruction of the lung by neutrophils remaining within the pulmonary microvessels. Extensive tissue damage leading to emphysema therefore would depend upon the extent of neutrophil recruitment and activation. In conclusion, in the present study of an in vitro mechanism which could lead to tissue damage and contribute to the pathogenesis of emphysema, it is apparent that activated PMNs play a highly important role in the development and progression of pulmonary emphysema.

References