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Basal secretion of lysozyme from human airways *in vitro*

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THE aim of this study was to examine the basal release of lysozyme from isolated human lung tissues. Measurements of lysozyme in the fluids derived from lung preparations were performed using a rate-of-lysis assay subsequent to acidification of the biological samples. Lysozyme released from bronchial preparations into fluids was greater than that observed for parenchymal tissues. The lysozyme quantities detected in bronchial fluids were not modified by removal of the surface epithelium. Furthermore, the quantities of lysozyme in bronchial fluids was correlated with the size of the bronchial preparations. These results suggest that the lysozyme was principally secreted by the human bronchi (submucosal layer) rather than by parenchyma tissues and that a greater release was observed in the proximal airways.

Key words: Lysozyme, Human bronchus, Parenchyma, Epithelium

Introduction

Lysozyme has been described to be present in glandular serous cells[1,2] and to be secreted by tracheal explants by both glandular and epithelial cells.[3] Furthermore, neutrophils and macrophages that accumulate within the airways may be also a source of lysozyme.[3,4]

Since high concentrations of lysozyme have been identified in airway lavage fluids[5,6] and in expectorated sputum from patients with asthma,[7] chronic obstructive lung disease[6–8] and cystic fibrosis,[8] the detection of this enzyme in biological fluids from patients with respiratory disease has frequently been monitored as an index of inflammation in human airways. However, in these investigations the origin of the lysozyme was not identified. Recently, Thompson and coworkers[6] determined the lysozyme concentrations in both bronchial and alveolar lavage fluid samples, but the relative contribution of lysozyme by proximal and distal airways was not discussed.

To investigate the possibility that this enzyme might serve as a useful marker of airways secretion and to examine the relative contribution of the upper and lower respiratory tract to lysozyme release from isolated human lung tissues, the basal release of lysozyme was measured in the biological fluids derived from proximal and distal bronchial rings, as well as from parenchymal tissues.

Material and methods

Functional studies

Human lung tissues were obtained from patients (six lung samples) who had undergone surgery for lung carcinoma. Tissues were obtained from the resected lung at a distance from the tumor area. Parenchymal lung tissues were cut as strips (2/15 mm). The subsegmental bronchi (first to third generation bronchi) were dissected free from parenchymal tissue, cut as rings of constant length (2 mm, internal diameter) and weighed. In some bronchial rings, the surface epithelium was removed by gently rubbing the luminal surface of the bronchial preparations with a moistened cotton swab. Bronchial and parenchymal tissues were washed with a physiological Tyrode's solution, pH 7.4 and allowed to equilibrate in Tyrode's solution for 1 h at 37°C in a humidified incubator (5% CO_2/air). Bronchial rings were placed in 1 ml of Tyrode's solution while parenchymal tissues were equilibrated in 10 ml of the Tyrode's solution. At the end of this equilibration period, media were exchanged. Bronchial and parenchymal tissues were replaced in identical volumes of fresh Tyrode's solution previously warmed for 1 h at 37°C. Media derived from bronchial and parenchymal tissues were collected and stored at –20°C.

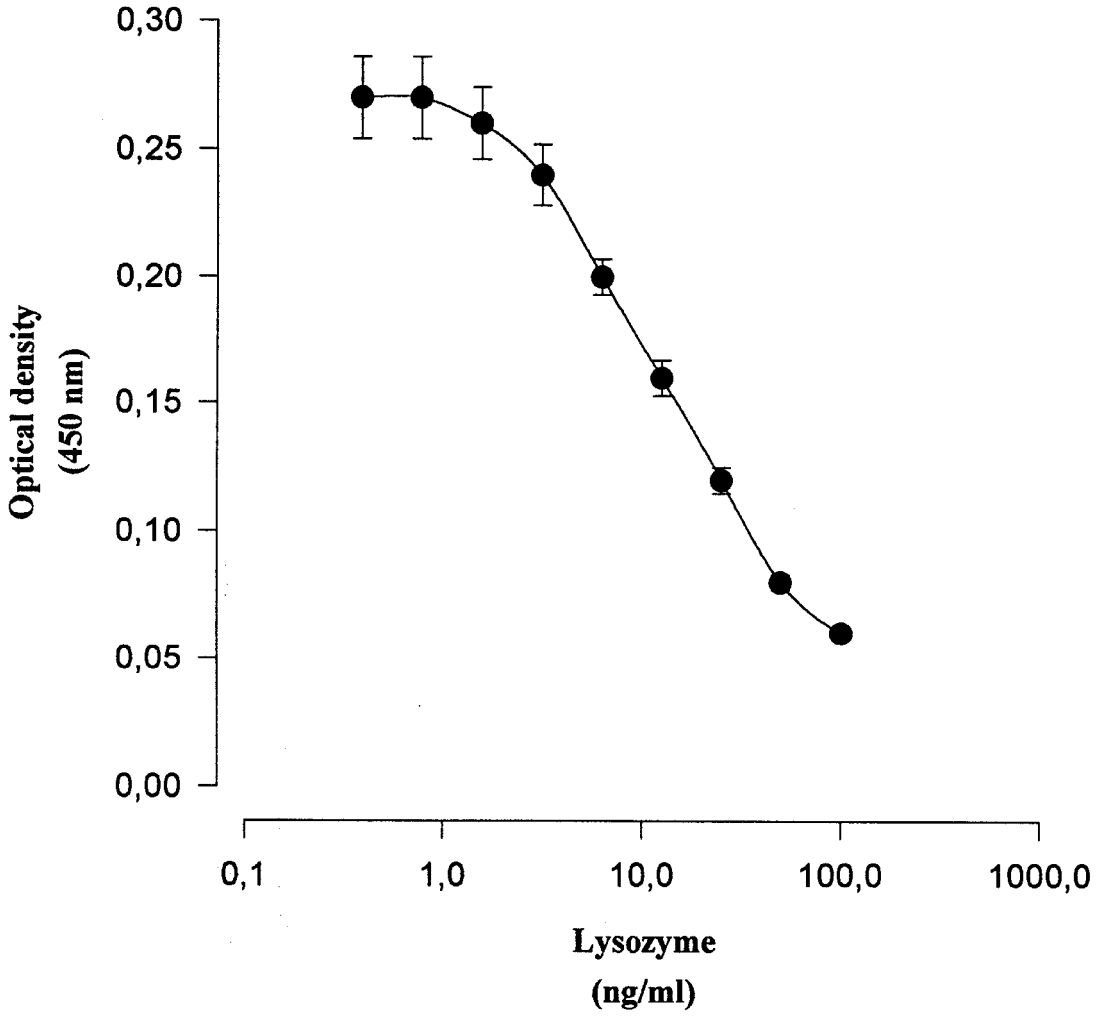

FIG. 1. Calibration curve of the rate-of-lysis assay based on the bactericidal activity of lysozyme. Data points represent the means±SEM (n=15) for optical density measured in triplicate at 450 nm at each egg-white standard concentration (0.5–100 ng/ml).

Assay of lysozyme

Lysozymal activity was determined spectrophotometrically by measuring the initial rate of lysis of a 3 mg/ml *Micrococcus lysodeikticus* cell wall suspension (Sigma Chemical Co., St Louis).[9] Standard curves were constructed by incubating egg-white lysozyme (0.5–100 ng/ml; Sigma Chemical Co.) in 1.5 ml of 50 mM potassium phosphate buffer, pH 7.4 containing *M. lysodeikticus* (300 µg/ml), sodium azide (0.1%; Sigma Chemical Co.), BSA (1 mg/ml; Sigma Chemical Co.), mixed and incubated at 37°C for 2 h. In parallel, fluids (1 ml) collected from bronchial and parenchymal tissues were diluted in deionized water (1:4 v/v) and acetic acid was added to adjust the pH to 4.5. Samples were heated at 100°C for 2 min[10] and centrifuged (3000 rpm for 10 min) to precipitate impurities. An aliquot of 200 µl of each treated sample was added to 1.8 ml of potassium phosphate buffer containing *M. lysodeikticus* (300 µg/ml), BSA (1 mg/ml), 0.1% sodium azide and incubated at 37°C for 2 h. Change in turbidity was monitored at a wavelength of 450 nm with potassium phosphate buffer as blank. No change of turbidity occurred under these conditions in absence of enzyme. A standard curve was plotted as optical density *versus* the concentration of lysozyme.

Calculation

Coefficients of variation of the lowest and the highest value of the standard curve were calculated by dividing the standard deviation by the mean and expressed as a percentage. The concentration of lysozyme detected in the fluids derived from human bronchial and parenchymal preparations are expressed as ng/ml and were estimated from the standard curves. All results are means± SEM. Statistical analysis were performed using the Student's t-test. A value (P< 0.05) was taken as an indication of significance.

Table 1. Effects of acidification and heating on the lysozyme detection

Bronchial fluids	Lysozyme (ng/ml)
Untreated	280±47
Acidified and heated	507±65*

Values are means±SEM (three preparations from three different lung samples; mean bronchial tissue wet weight: 900±30 mg).
*indicates that values are significantly different from untreated samples ($P<0.05$; Student's t test).

Results

The mean standard curve ($n=15$) for the lysozyme concentration measurements obtained with the egg-white lysozyme is presented in Fig. 1. The assay was sensitive since the linear part of the curve allowed the determination of lysozyme concentrations from 100 to 1.6 ng/ml. This assay was also highly reproductive since the coefficient of variation for the highest and the lowest value were 3% and 6%, respectively.

Whereas no significant alteration of the standard curve was observed when the egg-white lysozyme was acidified and heated (data not shown), the lysozyme concentrations determined in treated bronchial fluids were significantly increased compared with the contents of untreated fluids derived from the same bronchial preparations (Table 1).

The lysozyme quantities of fluids derived from bronchial rings were then compared with the concentrations detected in parenchymal fluids subsequent to acidification and heating of the samples. Approximatively two-fold higher concentrations were measured in bronchial fluids compared with the amounts detected in fluids derived from the parenchymal tissues (data not shown). Furthermore, the mean parenchyma wet weight was 19-fold higher than the bronchial wet weight, suggesting that the lysozyme quantities determined in treated bronchial fluids were largely dependent on the lung tissue wet weight from which samples derived.

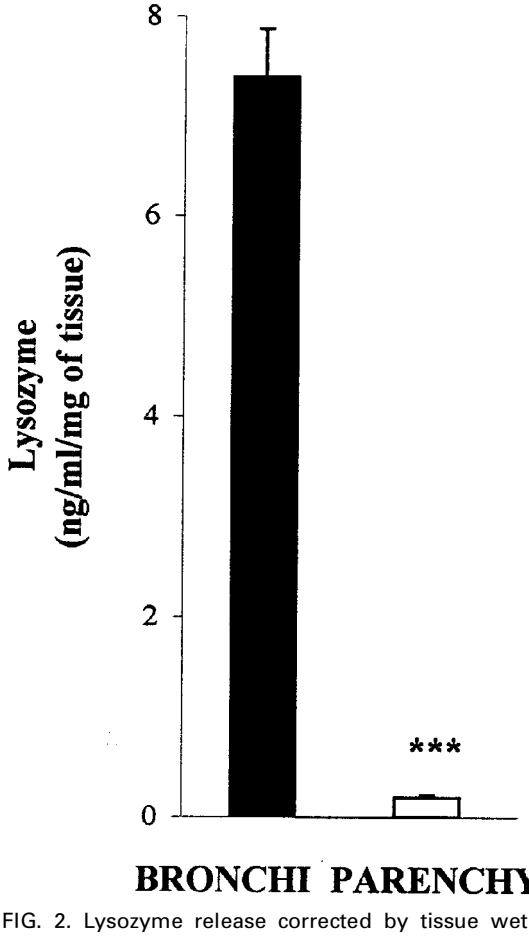

FIG. 2. Lysozyme release corrected by tissue wet weight and expressed as ng/ml/mg of tissue wet weight from human bronchial and parenchymal preparations. Values are means±SEM from 40 bronchial rings and 10 parenchymal preparations from six lung samples. ***indicates $P<0.005$ (Student's *t*-test).

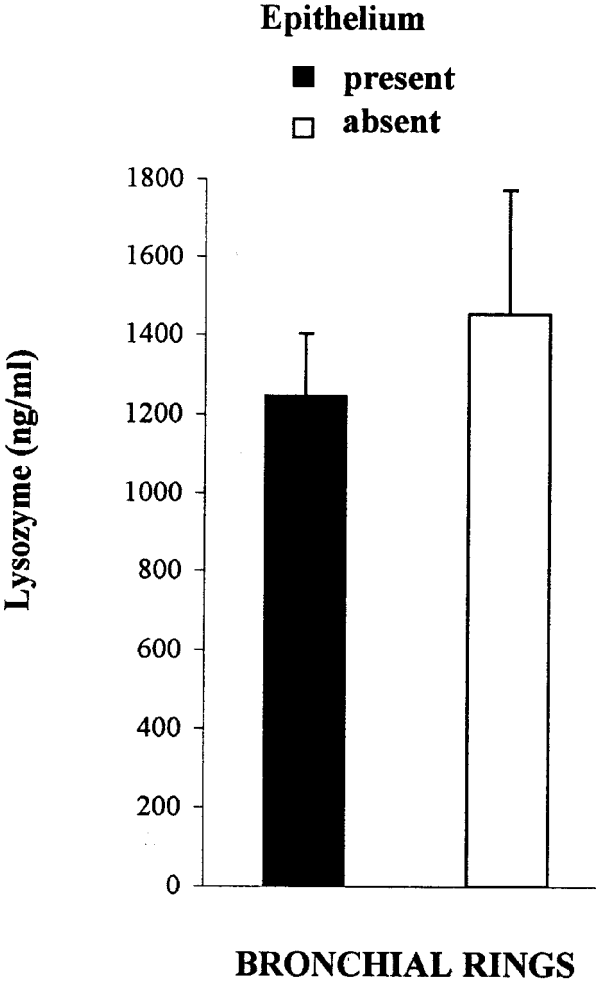

FIG. 3. Lysozyme quantities (ng/ml) contained in fluids derived from human bronchial preparations either with (■) or without (□) surface epithelium. Values are means±SEM (10–40 bronchial rings from three to six lung samples; mean tissue wet weight: 200±70 mg).

Since significant correlations were observed between the tissue wet weight and the quantities of lysozyme detected in fluids derived from either human bronchial rings (correlation coefficient, $r=0.86$; $P<0.05$) or lung parenchyma (correlation coefficient, $r=0.63$; $P<0.05$), normalization of the lysozyme fluid contents to tissue wet weight was performed. The lysozyme quantities detected in bronchial fluids were significantly higher (approximately, 15-fold) than the quantities of lysozyme detected in the parenchymal fluids (Fig. 2).

No significant difference in the lysozyme quantities was observed in the fluids collected from bronchial rings with an intact epithelium compared with the quantities detected in fluids derived from rings where the surface epithelium had been removed (Fig. 3).

Discussion

These results suggest that the measurements of lysozyme in the biological fluids derived from human bronchial and parenchymal tissues subsequent to acidification and heating facilitated the lysozyme detection. A predominent release of this enzyme from human bronchial rings, particularly from submucosal glands was observed, whereas lung parenchymal tissues released markedly less quantities of lysozyme. The results also suggested that proximal airways secreted higher lysozyme quantities than distal bronchi.

The determination of the lysozyme contents in fluids collected from human bronchial rings and parenchymal tissues was performed using a highly sensitive and reproductive spectrophotometric assay based on the bacteriolytic activity of this enzyme.[9,11] This assay allowed the detection of lysozyme levels as low as 1.6 ng/ml in biological fluids. However, strong interactions between the lysozyme and mucus glycoproteins have been reported due to their negative charge, leading to the aggregation of these proteins and to the difficulty in evaluating the lysozyme quantities exactly in biological samples.[12] Several years ago, Jollès and Petit[10] described a treatment of salivary fluids by acidifing and heating samples, which purified the enzyme without altering the lysozymal activity.[10,13] However, this observation has been largely neglected in the methods used to evaluate the lysozyme concentrations in biological samples. The present report clearly showed that the lysozyme quantities detected in the treated bronchial fluids were significantly increased compared with the contents of untreated samples. These data supported the notion that the lysosomal activity was stable after acidification and heating of samples and suggested that this treatment was suitable for purifying the enzyme and thereby facilitated the determination of the quantities detected by the rate-of-lysis assay.

The lysozyme quantities detected in human bronchial fluids were approximatively 1 µg/ml, whereas lower quantities of lysozyme (660 ng/ml) were detected in the fluids derived from parenchymal tissues (present report). Although high concentrations of this enzyme have been detected in airway lavages or nasal fluids from patients without respiratory distress (approximatively, 10 µg/ml),[5,6] the differences in the levels of lysozyme detected in bronchial fluids may be explained by the fact that measurements (present report) were performed in a model of isolated bronchial rings which were periodically rinsed with fresh medium. Previous investigations reported that the bronchial sample lavage fluid concentrations of lysozyme were higher than alveolar sample contents after normalization of the quantities to albumin.[6] In the present study, when the lysozyme quantities were corrected by tissues wet weight from which biological fluids were derived, bronchial contents were markedly higher (approximately, 15-fold) compared with the parenchymal fluid concentrations, supporting the previous observations. In human lung tissues, Klockars and coworkers[4] reported that alveolar epithelium was devoid of lysozyme while an intense lysozyme immunohistochemical labelling was observed in neutrophils and monocytes which accumulate within the airways. In view of these investigations, the present data suggested that the lysozyme detected in parenchymal fluids may be due to the degranulation of macrophages and neutrophils present in the lung connective tissue.

The present results showed that the lysozyme concentrations were highly correlated with the bronchial tissues wet weight, suggesting that the lysozyme source was greater in human proximal bronchi compared to the distal airways. Recent investigations reported that goat airway bronchioles and alveolar ducts were essentially constituted of six cell types, among which glandular structures were not present.[14] These data supported the notion that in the more distal portion of the lung, the glandular volume was smaller.

The present study also demonstrated that the removal of the surface epithelium from human bronchial rings did not modify the lysozyme release from these preparations. These data suggested that the submucosal layer was the principal source of lysozyme and supported previous immunohistochemical observations, which showed that the lysozyme was secreted from the bronchial glandular serous cells.[1,2]

In conclusion, the present report demonstrated that a treatment (acidification and heating) of biological fluids derived from lung samples facilitated the detection of lysozyme by the spectrophotometric rate-of-lysis assay. In addition, the measurements of lysozyme showed that this enzyme was released from human bronchial rings, specifically from glandular

cells of the submucosal layer rather than from lung parenchyma. Since various investigations reported that lysozyme levels were elevated in association with respiratory distress, such as chronic bronchitis and asthma[6–8] where glandular tissue volume was high, these data suggested that the lysozyme may be a marker of the secretory activity of glands and could be used in the study of agents regulating the glandular secretion.

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