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Effect of human leukocyte enzymes on tracheal mucosa and its mucociliary activity

Hans Tegner, Kjell Ohlsson, Nils Gunnar Toremalm and Claes von Mecklenburg, Malmö, Sweden.

Human neutrophil granulocytes contain proteolytic enzymes. In purulent bronchial and paranasal secretions these enzymes have been found extracellulary in complex with enzyme inhibitors as well as in free form indicating saturation of the inhibiting capacity.
Isolated human leukocyte enzymes, elastase and neutral protease, were found to arrest the mucociliary activity and subsequently cause superficial tissue destruction. Elastase was found to be the most potent of the enzymes. Experimental studies with elastase together with specific inhibitor indicated the importance of the enzyme inhibitors for the integrity of the mucous membrane.

INTRODUCTION

In acute purulent bronchitis bronchial secretion becomes proteolytic (Lieberman and Kurnick, 1963; Lieberman et al., 1965; Opie, 1905 and 1922; Ohlsson and Tegner, 1975). This proteolytic activity stems largely from leukocyte enzymes (Opie, 1905 and 1922; Lieberman and Kurnick, 1962 and 1963; Lieberman et al., 1965; Ohlsson and Tegner, 1975) occuring in abundance outside the cells and capable of breaking down elastin and collagen (Ohlsson and Tegner, 1975). Two of these enzymes, elastase and a collagenolytic neutral protease, have recently been isolated and characterized at the same time as specific antisera against them have been raised (Ohlsson and Olsson, 1973 and 1974). (For review and recent data concerning collagenolytic neutral protease see Ohlsson, 1979). The enzymes hydrolyse elastin, collagen and proteoglycans, which are all important tissue components (Ohlsson and Olsson, 1973, 1974).

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The potent enzymes are released *in vitro* in connection with phagocytosis of immune complexes (Ohlsson and Olsson, 1977) and *in vivo* in purulent bronchitis (Ohlsson and Tegner, 1975).

The availability of pure elastase and neutral protease has made it possible to study the effect of these enzymes on the function of the mucosal lining of the airways.

This paper concerns the effect of the above enzymes on the mucociliary activity of the tracheal mucosa *in vitro* and electron miscroscopic evaluation of the effect of the enzymes on the structure of the mucosa after varying time of exposure.

MATERIAL AND METHODS

Leukocyte enzymes. Human leukocyte enzymes, elastase and neutral protease, were isolated according to Ohlsson and Olsson (1974 and 1973).

Enzyme inhibitor. The low molecular weight synthetic elastase inhibitor N-acetyl-L-alanyl-L-alanyl-L-prolyl-L-valine chloromethyl ketone was obtained from Dr. James C. Powers, Georgia Institute of Technology, Atlanta, Ga., USA.

Elastase was incubated with this inhibitor in molar excess of about 100 times and dissolved in 0.1M Tris-HCl buffer, pH 7.4, with 0.15M NaCl for 15 min. at 24 °C, which resulted in complete inactivation of the elastase.

Measurement of enzyme activity. Elastase activity was determined on elastin-containing agarose plates according to the method described by Ohlsson and Olsson (1974).

Recording of mucociliary activity. Trachea from healthy rabbits was placed in an experimental chamber described by Håkansson and Toremalm (1965). The mucociliary activity was recorded according to a recently published method (Reimer et al., 1977). The tracheal preparation was rinsed for 10 min. in each one of three baths of 0.9% NaCl, after which the mucociliary activity was recorded on 3 occasions at 15 min. intervals and used as reference.

Exposure experiments. The tracheal preparation was incubated at 37 °C with the leukocyte enzyme elastase dissolved in 0.1M Tris-HCl buffer, pH 7.4, with 0.15M NaCl. The concentration of elastase varied between 200 and 1000 μg/ml. The neutral protease was dissolved in 0.1M Tris-HCl buffer, pH 8.3, with 0.01M $CaCl_2$ and 0.15M NaCl. The concentration of this enzyme varied between 500 and 1000 μg/ml.

The tracheal preparation was also incubated with inactivated elastase in the same concentration as active enzyme and under identical conditions.

In the control experiments the tracheal preparation was incubated with elastase buffer alone, with neutral protease buffer alone and with the above

mentioned low molecular weight synthetic elastase inhibitor dissolved in 0.1M Tris-HCl buffer, pH 7.4, with 0.15M NaCl.

During the experiment the incubation solutions were added so that they covered the surface of the mucosa. The solutions were aspirated at regular intervals to test the ciliary activity.

Electron microscopy. After conclusions of the mucociliary function tests the tracheal preparation was fixed and examined in transmitted light in the electron microscope and in the scanning electron microscope according to a method described by C. von Mecklenburg et al. (1974).

RESULTS

The leukocyte enzyme elastase can inhibit mucociliary activity. The inhibition varied with the concentration of the elastase as shown by the representative curves (Figure 1). After about 4 hours incubation of the tracheal preparation with elastase in a concentration of 250 μg/ml, all mucociliary activity ceased. When the concentration was doubled (500 μg/ml) it ceased already within about 2 hours. Higher concentrations inhibited all action within about 30 min. at the same time as the incubation solution became turbid owing to dissolution of the surface layer of the mucosa.

Enzymatically inactive elastase had no demonstrable effect on ciliary activity (Figure 1).

Also collagenolytic neutral protease had an inhibitory effect (Figure 2). After incubation for about 5 hours with this enzyme in a concentration of 500 μg/ml, tracheal mucosa showed no ciliary activity.

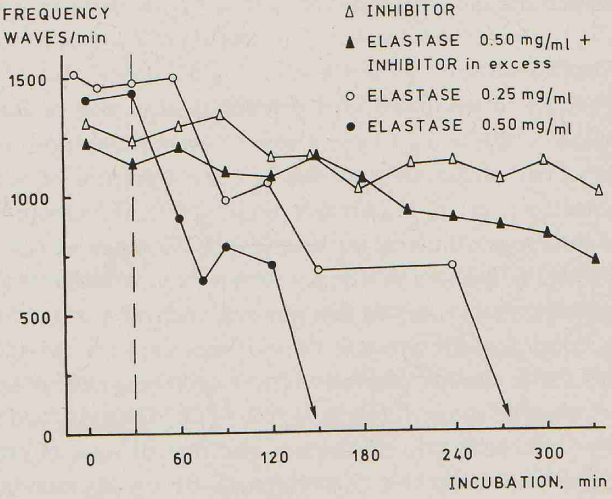

Figure 1.
Effect of active elastase on mucociliary activity (● and O, respectively) and of enzymatically inactive elastase complexed with the synthetic low molecular weight inhibitor (▲). Control experiment with inhibitor alone (△).

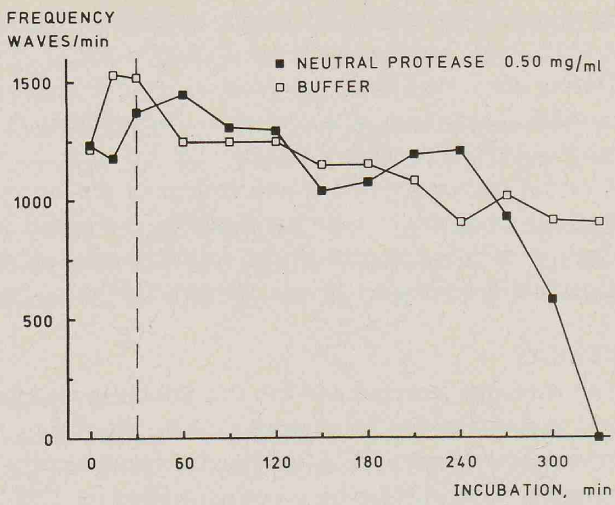

Figure 2.
Effect of collagenolytic neutral protease in a concentration of 500 µg/ml on mucociliary activity (■). Control experiment with buffer alone (□).

In control experiments with either inhibitor or enzyme buffers alone, ciliary activity was unaffected.

Electron microscopic examination of tracheal mucosa after the experiments revealed that both elastase and neutral protease damaged the mucosa. Elastase was the more injurious of the two enzymes and destroyed the entire mucosa, which no longer contained any ciliated cells (Figure 3). Beneath the apparently preserved basilar membrane, the mucosa was converted to a virtually amorphous mass with scattered collagen fibrils. Neutral protease damaged the cilia layer with destruction of the cilia and signs of degeneration of the underlying cells in the form of abundant vacuolisation and enlarged nuclei (Figure 4).

DISCUSSION

Interest in the activity of proteolytic enzymes in secretion from the airways was roused some 10 years ago by the observation of an association between inherited deficiency of the enzyme inhibitor α_1-antitrypsin and the early development of pulmonary emphysema (Eriksson, 1965). α_1-Antitrypsin is a potent inhibitor of various leukocyte enzymes (Ohlsson and Olsson, 1973, 1974). A relative deficiency of the enzyme inhibitors may also occur in acute purulent infections of the airways, such as acute purulent bronchitis. In such a condition the amount of enzymes exceeds the inhibiting capacity, which results in the occurrence of free active enzymes and explains the long known proteolytic activity (Opie, 1905, 1922; Ohlsson and Tegner, 1975).

The concentration of elastase and that of neutral protease in purulent bronchial secretions has been determined by Ohlsson and Tegner (1975). The

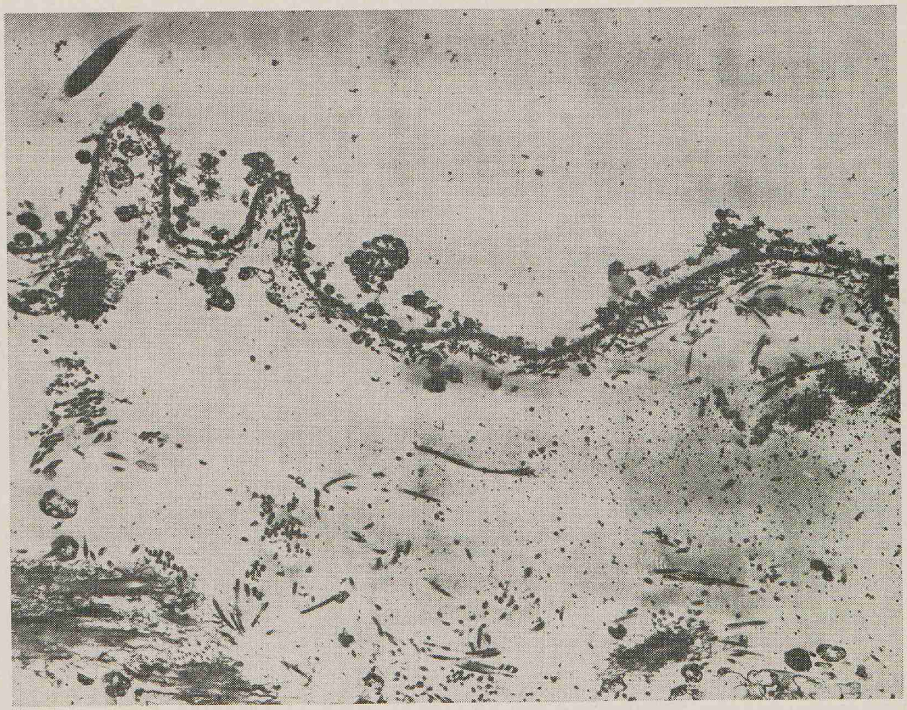

Figure 3. Electron microscopy in transmitted light of tracheal mucosa exposed to elastase in a concentration of 600 μg/ml for 6 hours.

availability of isolated enzymes has made it possible to assess also the effect of each of these enzymes on, among other things, the function and structure of the tracheal mucosa. The mucociliary action of the tracheal mucosa has proved a suitable indicator for the local effect of the actual enzymes.

Both of the above mentioned leukocyte enzymes inhibited the mucociliary activity of the trachea and at the same time destroyed the mucosa. Elastase appeared to be the more injurious enzyme. Both enzymes left the basilar membrane intact, an observation apparently explaining the rather quick regeneration of the mucosa *in vivo*.

Elastase seems to be the most interesting of the leukocyte enzymes from a pathogenetic point of view because of the role it plays in the destruction of cilia shown in this investigation and the development of pulmonary emphysema (Mittman, 1972).

According to our preliminary results, inactivated elastase did not arrest the mucociliary activity. This suggests that the enzymatic activity of elastase is responsible for the tissue damage mentioned above and at the same time underlines the importance of the protection offered by enzyme inhibitors.

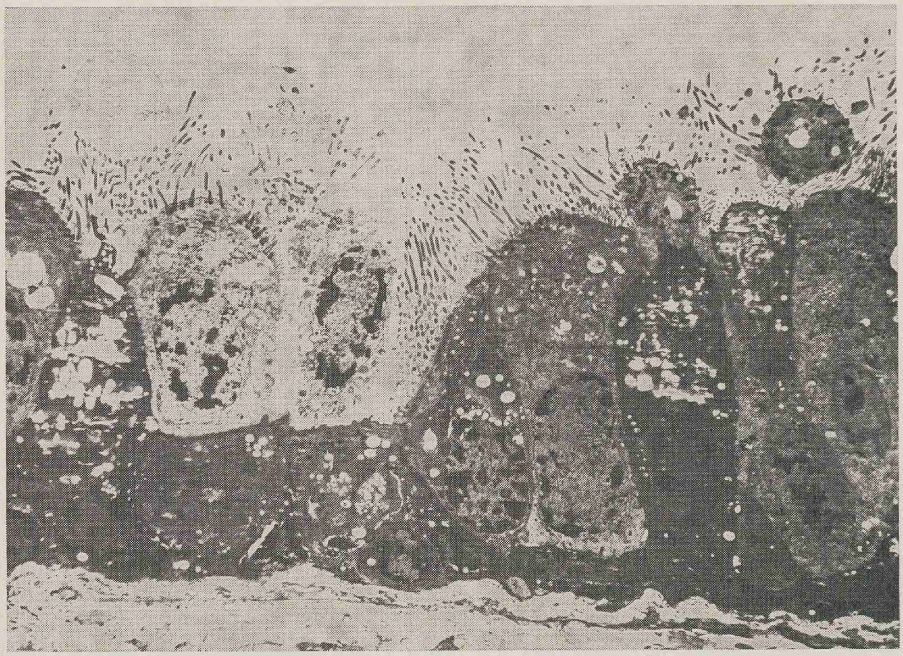

Figure 4. Electron microscopy in transmitted light of tracheal mucosa exposed to neutral protease in a concentration of 500 µg/ml for 5 hours.

Corresponding experiments on human mucosa are necessary before any definite conclusion can be drawn. The role played by the acid stable, low molecular inhibitor, antileukoprotease, present in the mucosa is of special interest, as it is a very strong inhibitor of granulocyte elastase (Tegner and Ohlsson, 1978). It would also be interesting to examine the biologic effect of enzyme activity on mucosal function in more or less closed cavities, such as paranasal sinuses and the middle ear, in chronic long-standing or recurrent infections.

ZUSAMMENFASSUNG

Menschliche neutrophile Granulocyten enthalten proteolytische Enzyme. In eitrigen Bronchial- und Nebenhöhlensekreten sind diese Enzyme extracellulär sowohl in Komplexen mit Enzyminhibitoren als auch in freier Form gefunden worden, was für die Sättigung des Inhibitorkapazität spricht.

In vorliegender Arbeit wurde gezeigt, dass isolierte menschliche Leukozytenzyme, Elastase und eine neutrale Protease, die schleimtransportierende Cilienaktivität hemmen und nach längerer Inkubationszeit eine oberflächliche Gewebedestruktion verursachen. Hierbei zeigte Elastase die höchste

Aktivität. Weitere Versuche mit Elastase und einem spezifischen Inhibitor weisen auf die Bedeutung der Enzyminhibitoren für die Integrität des Schleimhaut hin.

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Hans Tegner, M.D.
Kjell Ohlsson, M.D.
Nils Gunnar Toremalm, M.D.
Departments of Oto-Rhino-Laryngology and Clinical Chemistry
Malmö General Hospital
S-214 Ol Malmö, Sweden

Claes von Mecklenburg, Ph.D.
Zoological Institute
University of Lund
S-221 Ol Lund
Sweden