Protease and Antiprotease Activity in the Respiratory Secretions of Horses Suffering from Chronic Pulmonary Disease

Gabriele Grünig, M. Hermann and *R. von Fellenberg*

Division of Applied Veterinary Physiology of the Department of Veterinary Physiology and Veterinary Clinic of Internal Medicine, University of Zurich

Introduction

Until now the etiology and pathogenesis of the clinical syndrome "chronic pulmonary disease" of the horse is, for the most part, only poorly understood. In human medicine it is thought that free protease activity, a result of a protease to protease-inhibitor imbalance, might play an important role in chronic pulmonary diseases (*Janoff*, 1983).
This hypothesis arose in 1964 when *Laurell* and *Erickson* detected a high incidence of alveolar emphysema in human patients with inherited homozygous α_1 antitrypsin deficiency. *Gross* and his colleagues (1965) produced emphysema in experimental animals by intratracheal instillation of elastases. In smokers, bronchitis and emphysema are thought to be a result of proteolytic damage since cigarette smoke enhances the accumulation of protease producing cells, namely neutrophils and macrophages, in the airways (*Janoff*, 1983). In all these conditions, nonrestricted, free proteases are thought to occur. Elastases, which are special proteases, could destroy elastic tissue thus producing alveolar emphysema. Proteases may be involved in the pathogenesis of bronchitis and bronchiolitis by nonspecific irritation of the airways or by production of an inflammatory response (*Thomson* et *al.*, 1983).
Proteases can be of endogenous or exogenous origin. Endogenous proteases and elastases are produced by neutrophils. These neutrophil enzymes have been detected in several species including the horse (*v. Fellenberg* et *al.*, 1985). Other protease producing cells are macrophages (*Janoff*, 1983). Exogenous proteases stem from microorganisms: for example, the chymotrypsin-like proteases from Micropolyspora faeni (*Thomson* et *al.*, 1983) or elastases from several Bacillus and Streptomyces species (*Mandl* et *al.*, 1962).
The aim of this study was to examine the role of protease activity in the pathogenesis of chronic pulmonary diseases of the horse.

Materials and Methods

Respiratory secretions were examined for protease activity, cellular content and bacteria. The secretions were collected during fiberoptic endoscopy of 142 horses referred to the clinic because of chronic cough and/or poor performance. All the horses were thoroughly examined and whenever possible they were grouped according to the severity of their chronic lung disease: a) low grade, b) middle grade and c) high grade (Table 1).
The secretions were aspirated via a catheter protruded through the endoscope biopsy channel into a mucus pool in the trachea. No washes were performed in horses without secretions present in the trachea or with extremly viscous secretions because those washes could not be compared to the secretions obtained directly.
The bacteriological examinations were carried out by the Department of Veterinary Bacteriology, University of Zurich. Cytological examinations were carried out by one person in the laboratory of the Veterinary Clinic of Internal Medicine, University of Zurich. The smears of the respiratory secretions were stained with May-Grünwald Giemsa and the amount of the different cell types was graded from + + + +: very many to −: none. Protease activity was measured in supernatants (sol-phases) and sediments (gel-phases) of respiratory secretions which had been centrifuged for 45 minutes at 50,000 g. Sol and gel-phases were either examined immediately or frozen before use. Protease activity was measured by the release of a dye from the substrate hide powder azure®. The assay has been described in detail (*Grünig*, 1985). Respiratory secretions which yielded an extinction smaller than 0.1000 after incubation with hide powder azure® were considered to contain questionable protease activity because the blanks of this assay were only 2–3 times smaller than 0.1.
Elastase activity was measured by an elastin agarose diffusion technique (*v. Fellenberg* et *al.*, 1985).

Results

Protease activity was present in many respiratory secretions. First we wanted to know if the protease activity was evenly distributed between the sol and gel phases and if the destruction of all cells by a detergent (CTAB) would increase the protease activity of the gel phases. The sol phases had similar protease activity compared to their respective gel phases and gel phases treated by detergent did not differ from untreated gel phases in their protease activity as shown in Table 2. Therefore the sol phases were used in

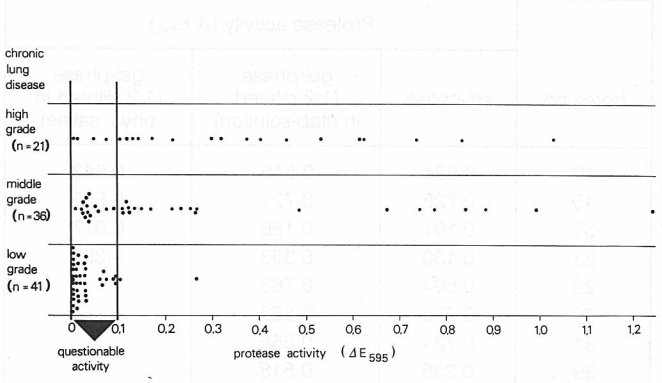

Fig. 1: Protease activity in respiratory secretions compared with the severity of the clinical signs. Each dot represents the result of one horse. The horses were grouped according to the severity of their chronic lung disease.

	chronic lung disease		
	low grade	middle grade	high grade
history	nasal discharge, coughing in the stable and/or at exercise, poor performance		
respiratory rate at rest	normal	sometimes elevated	elevated
type of respiration	slightly pronounced expiration	pronounced abdominal expiration	strongly pronounced abdominal expiration
auscultation after administration of lobelin®	moist rales	moist or dry rales, crepitation, wheezing	dry rales, crepitation, wheezing
PaO_2	around 90 mmHg	85 mmHg or lower	80 mmHg or lower
lung borders at percussion	normal	normal or slightly distended	usually distended
endoscopy: — discharge	small-moderate amount of liquid, transparent-white discharge	moderate-great amount of white-yellow discharge, either liquid and arranged in lakes or viscous	yellow discharge, either in moderate amounts and very viscous and adhesive, or in great amounts, liquid-viscous
— mucus membrane of trachea and proximal bronchi	normal	usually normal	proliferations
examination at exercise: — nasal discharge — cough — recovery	scant occasionally usually normal	moderate usually occasionally delayed	scant or copious always delayed

Table 1: Criteria for the classification of horses with chronic lung diseases (by Fischer, Diss. Hannover, 1980; modified)

further experiments with hide powder azure®. Protease activity in the respiratory secretions was correlated with the severity of the clinical symptoms (Figure 1). Horses with middle-grade and with high-grade disease contained more frequently protease activity in their respiratory secretions than horses with low-grade disease. This difference was highly significant ($p < 0.001$).

There was also a correlation between protease activity and the content of neutrophils present in the respiratory secretions. In Figure 2 the amount of neutrophils is plotted against the protease activity for each secretion. Secretions with + + + and + + + + neutrophils were by far more often proteolytically active than secretions with + + or less neutrophils. This difference was highly significant ($p < 0.001$). Protease activity was neither related to the other types of cells occuring in the secretions nor to bacterial growth. Elastase activity could not be detected in any of the secretions tested.

horse no	Protease activity (ΔE_{595})		
	sol-phase	gel-phase (1:2 diluted in ctab-solution)	gel-phase (1:2 diluted in phys. saline)
40	0.624	0.515	0.643
45	0.728	0.721	0.553
51	0.191	0.166	0.077
53	0.130	0.333	0.392
22	0.594	0.763	
30	0.775	0.883	
31	0.734	0.355	
39	0.235	0.518	
47	0.080	0.066	0.040

Table 2: Protease activity in sol- and gel-phases of respiratory secretions

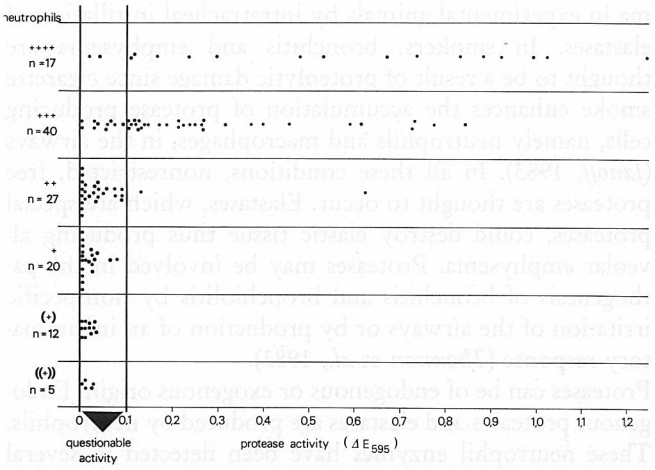

Fig. 2: Protease activity in respiratory secretions compared with the amount of neutrophils. Each dot represents the result of one horse. The horses were grouped according to the amount of neutrophils in their respiratory secretions.

The severity of the disease was compared with the cytology of the bronchial secretions. Middle-grade and high-grade diseased horses differed typically from horses with low-grade disease: Their respiratory secretions contained many more neutrophils and slightly fewer macrophages and epithelial cells than secretions of horses with low grade dis-

ease. All horses had only small quantities of lymphocytes, eosinophils and mast cells in their respiratory secretions. There was no correlation between the clinical diagnoses and the bacteria isolated, hence it is unlikely that the accumulation of neutrophils was due to bacterial infection on the average.

Discussion

Protease activity in respiratory secretions was very closely related to the clinical signs of horses with chronic pulmonary disease suggesting a role for protease activity in the pathogenesis of the disease. Nevertheless the mode of action of the protease is only poorly understood.

Protease activity and large numbers of neutrophils occurred simultaneously in the respiratory secretions. However, activity of protease inhibitors could also be detected along with protease activity in the same secretions (*Grünig*, 1984). It is known that many of these protease inhibitors are able to neutralize the neutrophil protease in vitro (*v. Fellenberg* et *al.*, 1985). Therefore it seems doubtful that protease activity of equine respiratory secretions originates from the neutrophils.

These findings could hint towards the role of protease in the pathogenesis of chronic pulmonary disease of the horse: Protease activity could enhance the accumulation of neutrophils either through the production of chemotaxins or through the direct display of chemotactic activity. Furthermore, proteases could irritate the airways especially in horses with very viscous secretions which stay in the airways for a considerable time. It is known that the release of mucins from goblet cells of rabbit tracheal epithelium is stimulated by a variety of proteases such as microbial proteases, pancreatic trypsin and pancreatic elastase (*Klinger* et *al.*, 1984). It is not known if the same is valid for equine goblet cells. Additionally some authors suggest that the action of proteases upon tracheal and bronchial epithelium might impair the ciliary function (*Wilson* et *al.*, 1985).

Elastase activity could not be demonstrated in equine respiratory secretions. Hence the role of elastases in the pathogenesis of alveolar emphysema in the horse remains obscure.

These findings are in contrast to the situation in man since neutrophil elastase activity could be detected in human sputum (*Burnett* and *Stockley*, 1980) or bronchoalveolar – lavage fluid (*Cochrane* et *al.*, 1983). Furthermore, there is no activity of protease inhibitors present along with protease or elastase activity in bronchial secretions of man (*Burnett* and *Stockley*, 1980).

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Dr. Gabriele Grünig
Veterinärphysiologisches Institut der Universität Zürich,
Winterthurer Straße 260,
CH-8057 Zürich, Switzerland