Concentrations of elastinolytic metalloproteinases in respiratory tract secretions of healthy horses and horses with chronic obstructive pulmonary disease

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Objectives—To determine whether samples of tracheal epithelial lining fluid (TELF) obtained from horses have elastinolytic activity characteristic of metalloproteinases, to compare elastinolytic activity in TELF obtained from healthy horses and horses with chronic obstructive pulmonary disease (COPD), and to determine whether chemically modified tetracycline-3 (CMT-3) inhibits elastinolytic activity in TELF.

Animals—10 horses with COPD and 10 healthy control horses.

Procedure—Zymography and fluorometry were used to measure elastinolytic activity, and EDTA was used to inhibit elastinolytic activity and verify that the activity was attributable to metalloproteinases. Possible inhibition of elastinolytic activity with CMT-3 was studied in vitro.

Results—Elastinolytic activity was found in TELF obtained from all horses, and this activity was significantly higher in TELF obtained from horses with COPD than in TELF obtained from healthy horses. For all samples, EDTA and CMT-3 inhibited elastinolytic activity.

Conclusions and Clinical Relevance—Elastinolytic activity is detectable in TELF obtained from horses and seems to be attributable to metalloproteinases. Elastinolytic activity in TELF is significantly inhibited by CMT-3. Elastinolytic activity in TELF can be detected by means of zymography or fluorometry. Increased elastinolytic activity may reflect destruction of pulmonary tissue in horses with COPD. Chemically modified tetracyclines such as CMT-3 may provide an additional treatment possibility for horses with COPD. (*Am J Vet Res* 2000;61:1067–1073)

Elastin, the major component of elastic fibers, is essential for proper structural and functional integrity of the lungs. Elastin, an insoluble hydrophobic molecule, is highly resistant to proteolysis. Degradation and improper repair of elastin are pivotal in development of pulmonary emphysema[1] and pulmonary dysfunction.

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Certain serine proteinases, cysteine proteinases, and **matrix metalloproteinases (MMP)** express elastinolytic activity. Neutrophil elastase is a serine proteinase with broad substrate specificity, including elastin. Cathepsin L, a cysteine proteinase, degrades elastin at acidic pH.[2] Among matrix metalloproteinases, MMP-12 (**macrophage metalloelastase [MME]**),[3] MMP-2 and -9 (gelatinase A and B),[4] MMP-3 and -10 (stromelysin-1 and -2),[5] and MMP-7 (matrilysin)[6] have the ability to degrade elastin.

Increased elastinolytic activity comprising serine proteinases and MMP has been identified in **bronchoalveolar lavage (BAL)** fluid of humans who smoke[7,8] or who have emphysema[9] or asthma.[10] Alveolar macrophages from humans with emphysematous lungs produce increased amounts of matrix-degrading enzymes that attack elastin and collagens.[11] **Tracheal epithelial lining fluid (TELF)** obtained from horses with **chronic obstructive pulmonary disease (COPD)** expresses MMP activity with characteristics of gelatinolytic[12,13] and collagenolytic activity.[14] To our knowledge, elastinolytic activity has not been reported in samples of equine pulmonary tissues or respiratory tract secretions.

Infiltrating inflammatory cells (neutrophils, monocytes, and macrophages), fibroblasts, other resident connective tissue cells, and epithelial cells secrete elastinolytic proteinases. In addition to neutrophil elastase, neutrophils contain another elastinolytic serine proteinase, proteinase 3.[15] These compounds are potential participants in destruction of lung tissue. In regard to gelatinases, MMP-9 is a secretory component of neutrophil primary granules and a major inducible macrophage product. Human mononuclear phagocytes express a changing profile of proteinases that participate in elastin degradation. Immature mononuclear phagocytes synthesize, store, and release neutrophil elastase, whereas mature monocytes store but do not synthesize that enzyme. After development to mature macrophages, they neither synthesize nor store neutrophil elastase[2]; instead, they express neutrophil elastase-like activity that can be inhibited by MMP inhibitors. As monocytes differentiate into macrophages, they lose the ability to secrete MMP-7, but they do acquire the capacity to produce large amounts of other MMP such as MMP-2, -9, and -12.[16] Macrophage MMP can directly degrade insoluble elastin.[3] Human alveolar macrophages also synthesize cysteine-type proteinases such as cathepsin L with elastinolytic activity.[2] Furthermore, MMP-2, another potentially elastinolytic metalloproteinase, is produced

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predominantly by fibroblasts and other resident connective tissue cells.[7]

In horses with COPD, there is prominent airway inflammation[17] characterized by recurrent hypersensitivity, mucus secretion, and bronchospasm resulting in airway obstruction and altered gas exchange.[18] An extensive increase in the number of neutrophils is found in respiratory tract secretions.[18] These inflammatory cells are a possible cellular source of elastinolytic proteinases.[19] It has been suggested[20-22] that other cells such as macrophages,[23] epithelial cells, and endothelial cells may be sources for elastinolytic activity in lungs of humans and rats.

The objective of the study reported here was to determine whether elastinolytic proteinases are detectable in TELF obtained from horses and whether COPD has an effect on the amount of activity. A fluorometric elastase assay was modified to measure TELF elastin degradation on microplates. Elastinolytic activity also was determined by κ-elastin zymography. Additionally, the effects of EDTA[a] and **chemically modified tetracycline-3 (CMT-3)**,[b] a drug that could potentially be used to inhibit and downregulate pathologically increased MMP at sites of inflammation,[24,25] on elastin degradation by TELF was studied.

Materials and Methods

Horses—Ten healthy control horses (4 mares, 4 stallions, and 2 geldings) between 3 and 16 years old (median, 9 years) and 10 horses with an acute episode of COPD (4 mares, 1 stallion, and 5 geldings) between 6 and 14 years old (median, 6 years) were used in the study. Samples of TELF were collected and used for zymography. In addition, 4 samples of TELF from healthy horses and 4 samples obtained from horses with COPD were randomly selected for fluorometric analysis. Healthy horses did not have a medical history or clinical signs of respiratory tract disease, and results of endoscopic examination of the nasal passages, larynx, and trachea in those horses were normal. Cytologic evaluation of tracheal secretions revealed only a small number of neutrophils (0 to 1 on a scale of 0 to 4). Horses with COPD had a history of recurrent periods of coughing, mucus secretion, and respiratory distress for several months preceding the initial examination. Physical examination of horses with COPD revealed coughing, labored breathing with abdominal effort, and wheezing or crackling sounds audible during auscultation. Endoscopy of the nasal passages, larynx, and trachea revealed an excessive amount of mucopurulent secretion that contained a high number of neutrophils (from 2 to 4 on a scale of 0 to 4). The WBC counts as well as hemoglobin and fibrinogen concentrations were within reference ranges for all horses.

Collection of TELF—A tracheal wash was performed for each horse by inserting a sterile catheter through the biopsy channel of an endoscope.[c] Ten milliliters of saline (0.9% NaCl) solution was infused into the middle ventral aspect of the trachea, and the pool of solution that formed in the lower ventral aspect of the trachea was aspirated immediately. Between 5 and 15 ml of tracheal wash fluid was recovered; a portion of each sample was submitted for cytologic evaluation, and the remainder was stored at –70 C until analysis. For determination of neutrophil count, air-dried smears were stained by use of a rapid-staining kit[d] and Wright stain[e] (2% solution in methanol) and examined at 200X magnification by an experienced faculty member who did not know the history of each horse. Overall cell density of the smear was graded from 0 to 4 (0 corresponding to 0 to 2 neutrophils in the observation field, 1 corresponding to a field with a mild increase in neutrophils but no aggregates, 2 corresponding to a field with moderate increase in neutrophils and aggregates, 3 corresponding to a field with abundant increase in neutrophils and aggregates, and 4 corresponding to a field full of neutrophil aggregates).[26,27] To correct dilution of TELF samples as a result of infused saline solution, urea concentrations in serum and recovered tracheal wash fluid were measured in parallel.[28] Dilution effect was calculated for each TELF sample by dividing the urea concentration in TELF by the urea concentration in serum.[29] Results were expressed in equal dilutions to original respiratory tract secretions.

Fluorometric elastase assay—A modified fluorometric elastase activity assay for microplate detection of elastinolysis in TELF was used.[30] The degradation of fluorogenic substrate elastin-fluorescein[f] in a 50 m*M* Tris[g]-HCl buffer (10 m*M* $CaCl_2$, 0.1*M* NaCl, 0.1% Brij-35, 0.02% NaN_3, pH 7.5) was expressed by the release of fluorogenic **fluorescein-isothiocyanate (FITC)** and monitored at 10-minute intervals, using a fluorometer[h] connected to a computer. The reaction was conducted in clear 96-well microplates[i] for 12 hours at 37 C. Fluorescence was measured at an excitation wavelength of 485 nm and an emission wavelength of 538 nm. All assays were performed in duplicate, and results were expressed as mean values. Corresponding blanks without sample were included to analyze spontaneous hydrolysis of the substrate and were subtracted to obtain net elastase activities.

Two separate pooled samples, both containing an equal amount of 4 TELF samples obtained from each of 4 horses affected with COPD, were prepared to assess suitable substrate concentration for the TELF elastase assay. Elastin-fluorescein substrate was dissolved in 50 m*M* Tris-HCl buffer to yield a final concentration of 2 mg/ml that was then used to make serial dilutions.

Determination of elastinolytic activity in TELF by fluorometry—Samples of TELF obtained from 4 healthy horses and 4 horses with COPD were used. Fluorometric measurements were performed at 2 intervals to avoid distortion of results as a result of evaporation. Immediately after mixing of a sample, buffer, and fluorogenic substrate, 1 portion of the mixture was pipetted into a microplate well, and fluorogenic activity was measured for a period of 6 hours. The resulting solution was incubated in a sealed tube on a shaker at 37 C for 66 hours. Thereafter, an aliquot of the incubated solution was pipetted into a microplate well, and the fluorogenic activity was measured for another period of 6 hours. The 66-hour incubation was used to compare the fluorogenic method to κ-elastin zymography, which requires a long incubation time to enable observable degradation of elastin. Three replicates of each sample were pipetted for both assays. After subtraction of the corresponding blank value, results were corrected, based on the urea concentration ratio.

Inhibition of elastinolytic activity on fluorometry—To determine effects of EDTA and CMT-3 on elastinolytic activity, fluorometry was performed as described previously, except that TELF samples were incubated for 1 hour at 37 C with 0, 1, or 2.5 m*M* EDTA or 0, 25, 50, or 150 μ*M* CMT-3 prior to fluorometry. Inhibition effect was determined for the first 6-hour period.

Determination of elastinolytic activity by zymography—In addition to the use of the fluorogenic elastase assay, elastinolytic activity was determined by zymography, using κ-elastin[j] as substrate; the procedure was performed essentially as described elsewhere.[4] Most diluted TELF samples were freeze-dried before the assay (2 samples from horses with COPD and all samples from healthy horses). Samples were centrifuged[k] for 4 minutes at 170 X g, and the

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supernatant was diluted to a standard dilution of 1:6 on the basis of the serum-to-tracheal fluid urea concentration ratio.

For zymography, samples were mixed 2:1 with sample buffer (118 m*M* Tris, 64 m*M* H_3PO_4,[l] 20% glycerol, 0.04 g of bromphenol blue/L,[m] and 6% **sodium dodecyl sulphate [SDS]**,[n] pH 6.8), and incubated for 2 hours at 20 C. Samples then were loaded into 12% SDS-polyacrylamide gels containing 1 mg of κ-elastin/ml as substrate. A previously stained high-range molecular weight standard,[o] standard suspension of lysed equine neutrophils (prepared as described elsewhere[13]), 350 ng of recombinant human MMP-9 (prepared as described elsewhere[31]), and 1.27 µg of recombinant human MMP-2 (prepared as described elsewhere[31]) were analyzed in parallel.

After incubating the gels for 66 hours, elastinolytic activity was determined by placing gels on an even-light translumination table and recording values with a charge-coupled device black-and-white video camera provided with a zoom kino-lens system, which was linked to an image-analysis and processing system.[p] Mean gray scale of the sample was determined (gray scale ranged from 0 to 255 pixels, with white being 255 pixels and black being 0 pixels). Densitometric results were calculated in area mode after subtraction of background gray values.

Inhibition of elastinolytic activity on zymography—To determine the inhibitory effects of EDTA on elastinolytic activity, zymography was performed as described previously, except that 10 m*M* EDTA was added to the preincubation sample buffer. To remove SDS after zymography, gels were washed 3 times (10 min/wash) in 2 wash solutions. The initial wash solution consisted of 50 m*M* Tris, 0.02% NaN_3, and Tween-80 containing 10 m*M* EDTA, and the second wash solution was the same mixture supplemented with 1 µ*M* $ZnCl_2$ and 5 m*M* $CaCl_2$. Gels were then incubated in 50 m*M* Tris, 0.02% NaN_3, 1 µ*M* $ZnCl_2$, 5 m*M* $CaCl_2$, and 10 m*M* EDTA for 66 hours at 37 C.

To determine the inhibitory effects of CMT-3 on elastinolytic activity, zymography was performed as described previously, except that TELF samples initially were incubated for 1 hour at 37 C with 0, 50, 150, or 500 µ*M* CMT-3 before incubation with sample buffer and zymography. To remove SDS after zymography, gels were washed 3 times (10 min/wash) in 2 wash solutions. The initial wash solution consisted of 50 m*M* Tris, 0.02% NaN_3, and Tween-80, and the second wash solution was the same mixture supplemented with 0, 50, 150, or 500 µ*M* CMT-3. Gels then were incubated in a solution of 50 m*M* Tris and 0.02% NaN_3 (supplemented with 0, 50, 150, or 500 µ*M* CMT-3) for 66 hours at 37 C.

Statistical analyses—Medians and ranges were used to express dispersion. Differences between healthy horses and horses with COPD in regard to elastinolytic activity in TELF

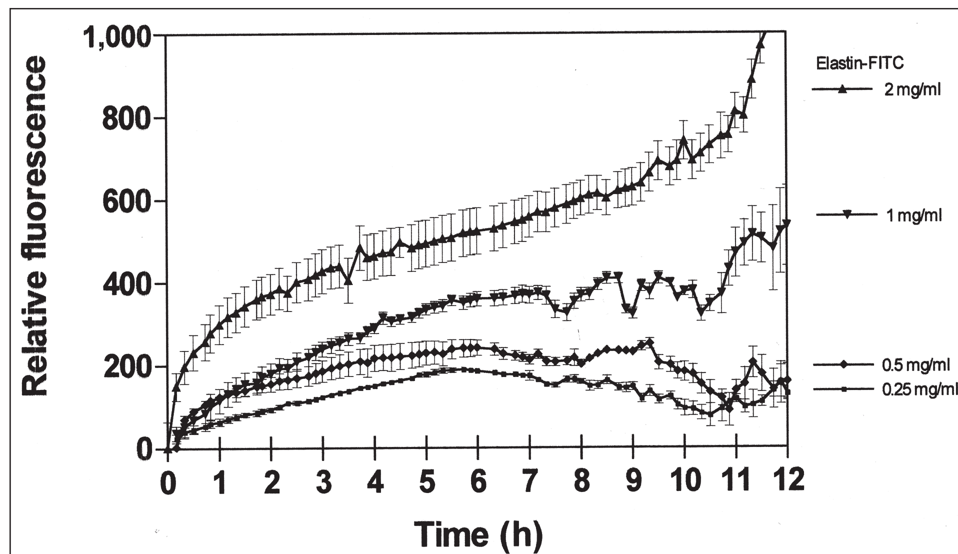

Figure 1—Results (mean ± SEM) for the serial dilution of the substrate elastin-fluorescein isothiocyanate conjugate (FITC). Assays were conducted on 2 pooled tracheal epithelial lining fluid (TELF) samples, each of which contained TELF from 4 horses with chronic obstructive pulmonary disease (COPD).

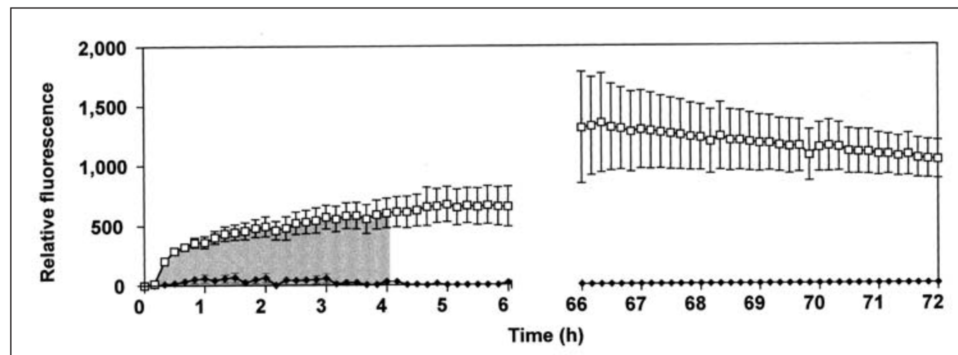

Figure 2—Results (mean ± SEM) for fluorometric determination of elastinolytic activity in TELF obtained from 4 healthy control horses (◆) and 4 horses with COPD (□). Shadowed area represents area under curve used for statistical analysis.

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was tested by use of the nonparametric Mann-Whitney test. Effects of inhibitors on the fluorometric assay were studied during the first 4 hours and were mathematically expressed as area under the curve, which were tested by use of the paired *t*-test. For comparison of elastinolytic activities with the fluorometric and zymographic assays as well as to correlate neutrophil scores with elastinolytic activity on fluorometry and zymography, a 2-tailed Pearson linear regression analysis was used. A value of $P < 0.05$ was considered significant. A software package[q] was used to conduct all analyses.

Results

Sample collection—Percentage of recovered tracheal wash fluid that was TELF ranged from 1.15 to 13.11% (mean, 4.42%; median, 3.10%) for healthy horses. For horses with COPD, the percentage of tracheal wash fluid that was TELF ranged from 17.12 to 76.34% (mean, 48.54%; median, 43.75%).

Fluorometric assay—Determination of elastinolytic activity from TELF was optimal at 2 mg of substrate/ml (**Fig 1**). Only results obtained during the initial 6 hours in both measurement periods were used, because decrease of sample volume as a result of evaporation started to disturb fluorescence values after 6 hours of incubation in the fluorometer. This was detectable as variable fluorescence values, especially at lower substrate concentrations.

Fluorometric determination of elastinolytic activity—Elastinolytic activities in TELF obtained from horses with COPD and healthy horses had significant differences during the 6-hour measurement period (**Table 1**). When area under the curve was used to study differences of elastinolytic activities during the first 4 hours of the measurement period, significantly ($P = 0.028$) higher values in TELF from horses with COPD (range 1,233 to 2,583; median, 1,626) than in TELF from healthy horses (range 0 to 279; median, 119; **Fig 2**) were detected. Elastinolytic activity in TELF from horses with COPD after 66 hours of incubation ranged from 442 to 2,467 (median, 1,207); however, elastinolytic activity was not detectable in TELF from healthy horses after 66 hours of incubation. Elastinolytic activity determined by use of fluorometry correlated significantly ($R = 0.966$, $P < 0.001$) with neutrophil score in TELF.

Zymographic determination of elastinolytic activity—Analysis of results of zymography revealed that TELF degraded κ-elastin. Elastinolytic activity was significantly ($P < 0.001$) higher in TELF from horses with COPD (range, 567 to 2,971; median, 1,144) than that in TELF from healthy horses (range, 37 to 233; median, 104; **Fig 3**). Most activity on gels was expressed at 80 to 95 kd, which was equal to the activity of pure human MMP-9. Pure human MMP-2 was expressed at 72 kd, but corresponding activity was not found for TELF at that molecular weight (**Fig 4**). Slight activity in TELF of horses with COPD was found at 25 kd. Equine blood neutrophil lysate revealed elastinolytic activity at the same molecular weight as for pure human MMP-9. Results of zymography revealed that elastinolytic activity in TELF correlated significantly ($R = 0.708$, $P < 0.001$) with neutrophil score in TELF.

After 66 hours of incubation, elastinolytic activity in TELF samples determined by use of zymography and fluorometry correlated significantly ($R = 0.868$, $P = 0.005$).

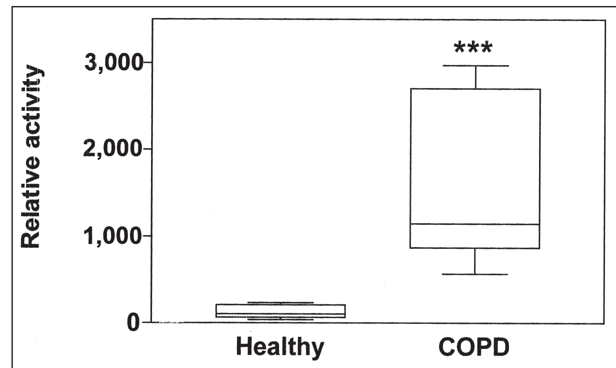

Figure 3—Results for κ–elastin zymography of elastinolytic activity in TELF obtained from 10 healthy horses and 10 horses with COPD after a 66-hour incubation. ***Values differ significantly ($P < 0.001$) between groups. Boxes represent 25th and 75th quartiles, error bars represent range of all values, and horizontal line within the box represents median value.

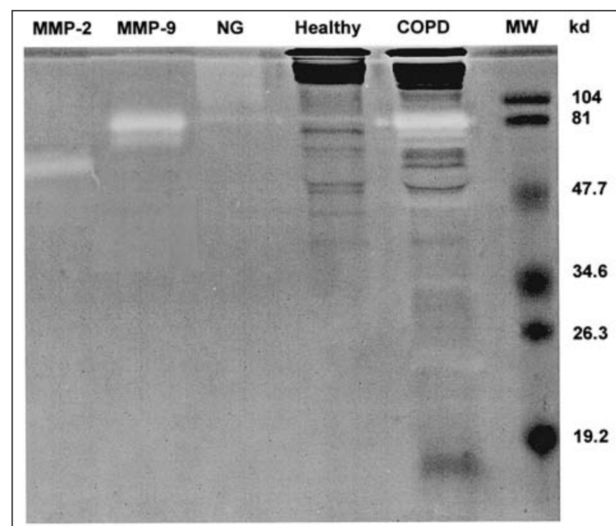

Figure 4—Results for κ–elastin zymography of elastinolytic activity. MMP-2 = Recombinant human matrix metalloproteinase (MMP)-2. MMP-9 = Recombinant human MMP-9. NG = Equine blood neutrophil lysate. Healthy = TELF from a healthy horse. COPD = TELF from a horse with COPD. MW = Molecular weight standards.

Table 1—Elastinolytic activity in tracheal epithelial lining fluid obtained from healthy horses and horses with chronic obstructive pulmonary disease (COPD), as determined by use of fluorometry

Time (h)	Healthy (n = 4)	COPD (n = 4)
0	0 (0–0)	0 (0–0)
1	116 (0–118)	429 (298–509)*
2	87 (0–165)	455 (339–734)*
3	68 (0–165)	507 (384–849)*
4	47 (0–68)	555 (368–949)*
5	0 (0–0)	618 (397–1,078)*
6	39 (0–78)	583 (360–1,102)*

Values reported are median (range).
*Values differ significantly ($P < 0.05$) from values for healthy horses.

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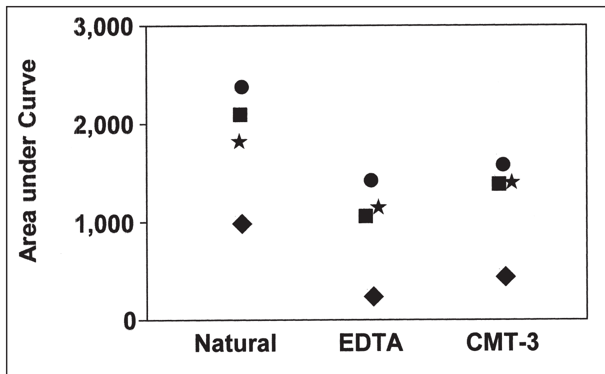

Figure 5—Inhibitory effects of EDTA and chemically modified tetracycline-3 (CMT-3) on elastinolytic activity (expressed as area under the curve) in TELF obtained from 4 horses with COPD. Results were determined by use of fluorometry during 4 hours. Natural = Activity without inhibitor. EDTA = Activity after incubation with 2.5 m*M* EDTA. CMT-3 = Activity after incubation with 25 µ*M* CMT-3. Each of the 4 horses is indicated by a unique symbol.

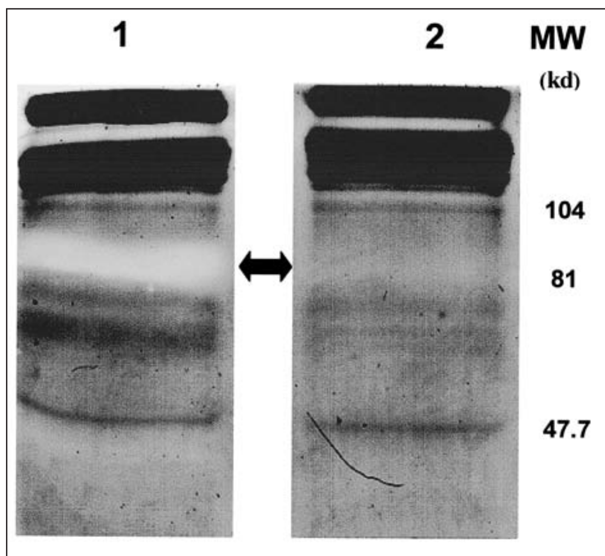

Figure 6—Results for κ-elastin zymography of elastinolytic activity in TELF obtained from a horse with COPD without inhibitor (lane 1) and after addition of 500 µ*M* CMT-3 (lane 2). Notice the area of elastinolytic activity inhibited by CMT-3 (arrow). MW = Molecular weight.

Inhibitory effects of EDTA—Analysis of results of fluorometry revealed that 1 m*M* EDTA inhibited elastinolytic activity (range, 18 to 54%; median, 31%) and 2.5 m*M* EDTA inhibited elastinolytic activity (range, 20 to 70%; median, 42%) in TELF samples from horses with COPD (**Fig 5**). Inhibitory effects were significant ($P = 0.002$ for 1 m*M* EDTA and $P = 0.015$ for 2.5 m*M* EDTA) for EDTA. Higher concentrations of EDTA interfered with the substrate. Results of zymography revealed that elastinolytic activity in TELF was completely inhibited by 10 m*M* EDTA.

Inhibitory effects of CMT-3—Using the fluorometric assay, 25 µ*M* CMT-3 significantly ($P = 0.004$) inhibited (range, 11 to 39%; median; 26%) elastinolytic activity in TELF samples from horses with COPD (Fig 5). Higher CMT-3 concentrations interfered with the substrate. For the zymographic assay, elastinolytic activity was totally inhibited by 500 µ*M* CMT-3 (**Fig 6**), and visible inhibition was observed with 150 µ*M* CMT-3.

Discussion

Using κ-elastin zymography and fluorescein-labeled elastin fluorometry, we detected elastinolytic activity in respiratory tract secretions of horses. Markedly increased elastinolytic activity in TELF from horses with COPD was observed for both methods, suggesting participation of elastases in destruction of lung tissue in horses with COPD.

Increased elastinolytic activity traditionally has been considered to be the main cause for proteinolytic lung destruction in several chronic lung diseases.[32] Increased amounts of elastinolytic activity have been detected from human respiratory tract secretions in connection with smoking and α_1-antitrypsin deficiency.[1,7] Increased metalloproteinase-type activity was first detected in humans with idiopathic pulmonary fibrosis[33] and subsequently was reported in connection with other respiratory tract diseases such as emphysema and asthma.[9,10]

In addition to having collagenolytic and gelatinolytic activity, metalloproteinases can degrade elastin. In addition to direct degradation of elastin, elastases have a role in biological cascades that result indirectly in degradation of elastin. Neutrophil-derived elastase is one of the pathophysiologic activators of MMP-9[34] present in TELF from horses with COPD.[35] Furthermore, MME, which is found in several animal species, promotes elastinolysis by cleaving α_1-antiproteinase and, thus, destroying the body's endogenous neutrophil anti-elastase shield.[2]

In horses with COPD, collagenolytic- and gelatinolytic-type metalloproteinase activity can increase in respiratory tract secretions.[12,13,36] Attempts to detect elastinolytic activity in respiratory tract secretions of horses with chronic pulmonary disease have not been successful,[37] although elastinolytic activity has been detected in equine neutrophils.[19]

Human MMP-2 and MMP-9 are elastinolytic[4] and, as indicated also by the results of the study reported here, are detectable by κ-elastin zymography. In our study, analysis of results of zymography revealed that the main elastinolytic activity was found at the same molecular weight as that for pure MMP-9, but activity was not found at the same molecular weight as that for pure MMP-2, indicating that the main elastinolytic activity seen on zymography was evidently attributable to MMP-9. Inhibitory effects of EDTA verified that all detected activity in TELF from horses with COPD, as determined by use of κ-elastin zymography, was attributable to metalloproteinase activity.

Equine blood neutrophil lysate expressed elastinolysis only at the same molecular weight as that for pure MMP-9, indicating that neutrophils are a potential source for elastases in TELF. Equine neutrophils apparently produce MMP-9 but not MMP-2,[13] which is similar to the situation for human neutrophils.[38] Equine neutrophils produce elastinolytic neutrophil elastase and neutral proteases, although these activities were one-fifth (for equine neutrophil elastase) and half (for

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neutral proteinases) those for human neutrophils.[19] Total elastinolytic activity with both assays correlated with neutrophil score in TELF samples, which further supported the fact that neutrophils are a predominant source of elastinolytic activity. Macrophages, epithelial cells, and connective tissue cells are other possible sources for elastinolytic activity. Shapiro et al[16] estimated that human MME and MMP-9 each accounted for approximately one-half of macrophage-derived metalloelastase activity in cultured alveolar macrophages. Additionally, it has been documented in vitro that alveolar epithelial cells synthesize several MMP.[21] The 25-kd elastinolytic activity in samples from horses with COPD may represent MME or neutrophil elastase, both of which cleave insoluble elastin.[39]

The fluorometric method measures total elastinolytic activity without separating specific enzymes; it includes neutrophil elastase, human MME, and other elastinolytic MMP.[39] The EDTA inhibited up to 70% of elastinolytic activity in TELF during fluorometric assay. Therefore, at least in part, the activity measured from TELF was attributable to MMP. Corresponding inhibition of human BAL fluid with metalloproteinase inhibitors, including EDTA, have been reported, using fluorescein-labeled substrate.[40] Analysis of results of the fluorometric elastase assay indicated a clear difference in TELF elastinolytic activity between healthy horses and horses with COPD. The differences in elastinolytic activity in TELF were evident within 1 hour after initiation of incubation, and the use of longer incubation times made the difference even more obvious (Table 1, Fig 2). Despite the small number of samples studied by use of fluorometry, there seemed to be a specific inhibitory value for each sample; these results resemble previous findings of caseinolytic activities in TELF[36] and suggest a mixed type of elastinolysis, which varies among horses. These and previous findings suggest involvement of other elastases in addition to metalloproteinases, all of which can be detected by use of the fluorometric method. The fluorometric assay has potential to be used as a diagnostic method to distinguish healthy horses from horses with respiratory tract disease. The method is faster than the zymographic method and enables clinicians to study the proteinolytic activity of a high number of samples at the same time.

Regarding the therapeutic implications of blocking the pathologically excessive proteinolytic cascades, especially in tissue-destructive inflammatory diseases, it has been reported[41] that tetracyclines inhibit the catalytic activities of mammalian MMP, collagenases, and gelatinases. Because long-term administration of tetracyclines to horses is not possible, we tested CMT that lack antimicrobial properties but still are able to inhibit mammalian MMP.[24] In the study reported here, CMT-3, which is the most effective tetracycline-derived MMP inhibitor,[25] inhibited all elastinolytic activity in TELF, as determined by use of zymography, and reduced correspondent elastinolytic activity, as determined by use of fluorometry. Inhibitory effects of CMT-3 on elastin zymography were similar to those previously found when gelatin was used as a substrate.[35] Furthermore, CMT-3 can inhibit human MMP-9.[25]

In the study reported here, we clearly documented that elastinolytic activity was detectable in respiratory tract secretions of horses, and MMP-type elastinolysis was increased in horses with COPD; thus, this suggests that excessive elastinolysis, evidently overcoming the endogenous antiproteinase shields, is involved in the pathogenesis of COPD in horses. Analysis of our findings further suggests that MMP-9 and its activation play a role in COPD in horses that is similar to that found for asthma in humans, a similar obstructive lung disease.[10] In a model of asthma in mice, MMP were suggested to be crucial for the infiltration of inflammatory cells and the induction of airway hyperresponsiveness.[42] Apparently, MMP also are crucial in elastinolysis, similar to their role in gelatinolysis, collagenolysis, and caseinolysis.[13,14,36] It has been suggested that recombinant elastase inhibitors such as horse blood leukocyte inhibitor could be used in treatment of emphysema in horses.[43] A synthetic MMP-inhibitor was observed to inhibit cellular infiltration to the airway lumen in an asthma model in mice.[42] Analysis of those results indicates that a targeted and efficient MMP inhibitor such as CMT-3 may be a valuable drug for use in diminishing elastinolytic activity in the respiratory tract of horses with COPD, which may enable inclusion of such inhibitors in the treatment of affected horses.

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[a]Metastat, CollaGenex Pharmaceuticals Inc, Newtown, Pa.
[b]Article 8418, Merck, Darmstadt, Germany.
[c]Type IB, 140 cm in length, Olympus Optical Co, Tokyo, Japan.
[d]Hemacolor No 16661, Merck, Heidelberg, Germany.
[e]No 34080, BDH Chemical LTD, Poole, UK.
[f]No E80, Elastin Products Co, Owensville, Mo.
[g]T-1378, Sigma Chemical Co, St Louis, Mo.
[h]Fluoroscan II, Labsystems, Helsinki, Finland.
[i]Cliniplate, Labsystems, Helsinki, Finland.
[j]No KE57, Elastin Products Co, Owensville, Mo.
[k]Biofuge A, Heraeus, Sepatech, Germany.
[l]Article 573, Merck, Darmstadt, Germany.
[m]Article 8122, Merck, Darmstadt, Germany.
[n]No 44244, BDH Chemicals Ltd, Poole, UK.
[o]No 161-0309, Bio-Rad, Richmond, Calif.
[p]Cream, Kem-En-Tek, Copenhagen, Denmark.
[q]Prizm II, Graph Pad Software Inc, San Diego, Calif.

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