Alveolar clearance in horses with chronic obstructive pulmonary disease

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Objective—To assess sensitivity of scintigraphic alveolar clearance rate as an indicator of alveolar epithelium damage in horses.

Animals—5 healthy horses (group A) and 5 with chronic obstructive pulmonary disease (COPD; group B).

Procedure—Horses underwent clearance rate (k [%/min]) determination. Clearance rate of group-B horses was determined after remission of the disease following 2 months at pasture (remission 1), stabling in a controlled environment (remission 2), and during crisis induced by exposure to moldy hay and straw. Methacholine challenge test was performed at each investigation period to determine nonspecific pulmonary airway hyperresponsiveness. Pulmonary function tests (PFT) also were performed, and cell populations in bronchoalveolar lavage (BAL) fluid were determined on another occasion.

Results—Group-B horses had significantly faster mean clearance rate during crisis (k = 4.30 ± 0.95%/min), compared with that for remission 1 (k = 1.98 ± 0.55%/min), which did not differ from the rate in group-A horses (k = 1.95 ± 0.33%/min). Despite lack of clinical signs of COPD during remission when stabled in a controlled environment, an intermediate value was found (k = 3.20 ± 0.72%/min).

Conclusions—This technique allowed grading of lung damage induced by COPD, whereas use of PFT and determination of BAL fluid cell populations failed to differentiate between remission 1 and remission 2.

Clinical Relevance—Determination of alveolar clearance rate by use of scintigraphy is a sensitive indicator of lung damage. A modified clearance rate was found despite the lack of clinical and functional changes. (*Am J Vet Res* 1999;60:495–500)

A scintigraphic method has been described to noninvasively measure pulmonary epithelial permeability as an index of alveolar epithelium damage in horses.[1] This method relies on the hypothesis that hydrophilic low molecular weight **^{99m}Tc-labeled diethylene triamine penta-acetate** (**^{99m}Tc-DTPA**) chelate, deposited in alveolar regions by nebulization, slowly diffuses through the tight intercellular junctions of the alveolar-capillary barrier. Because tight intercellular junctions of the alveolar cells are tighter than those of the capillary endothelium,[2] speed of disappearance of tracer from the lungs depends mainly on the alveolar epithelium. If an infectious or noninfectious disease affects the alveolar epithelium, clearance of radiolabeled DTPA from the alveoli to the perfusing blood will be accelerated.[3]

Using this technique, the authors reported that horses with **chronic obstructive pulmonary disease** (**COPD**) in acute crisis had significantly faster ^{99m}Tc-DTPA clearance rates than did healthy horses.[1] Acute expression of the disease is a hypersensitive response[4] of the respiratory system to spores inhaled from moldy hay and straw.[5-7] Bronchospasm, excess mucus secretion, and inflammation of the airway characterize this disease.[8] Furthermore, the airways are nonspecifically hyperresponsive, as evaluated by histamine[6,9] or **methacholine** (**MCh**)[10] challenge tests. The inflammatory process is accompanied by increased numbers of neutrophils in **bronchoalveolar lavage** (**BAL**) fluid,[1,7,11-16] which may potentially cause structural alterations in tight intercellular junctions of alveolar cells because of proteases, reactive oxygen species, and inflammatory mediator release.[17-19] Damages to tight intercellular junctions are believed to cause enhanced permeability.

Remission of clinical signs of COPD with restoration of normal **pulmonary function test** (**PFT**; eg, mechanics of breathing and blood gas analysis) results and disappearance of airway hyperresponsiveness are observed after a period at pasture[6,9,20-24] (ie, an allergen-free environment). Thus, stabled horses with COPD require particular care regarding forage and bedding to remain in clinical remission. Feeding of grass silage and use of wood shavings as bedding are often recommended[25,26] as replacements for hay and straw. While they are in this controlled environment,[24,27] horses with COPD are maintained free of signs of the disease and have normal PFT results; however, nonspecific airway hyperresponsiveness exists, and the reactive state of the bronchi is intermediate between values obtained at pasture and during acute crisis.[10]

Analysis of ^{99m}Tc-DTPA lung clearance is believed to be a sensitive test for studying early changes in alveolar epithelial permeability.[3,28,29] The study reported here was designed to determine whether this technique enables detection of a subclinical inflammatory process, confirmed by results of MCh challenge tests, by assessing ^{99m}Tc-DTPA clearance rate of horses with COPD after 2 consecutive 60-day periods (at pasture and stabled in a controlled environment[24] [low in

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aeroallergens and well ventilated]) and during acute exacerbation of the disease induced by natural challenge (ie, moldy hay and straw).

Materials and Methods

Horses—Two groups of 5 horses were used in a ^{99m}Tc-DTPA clearance study. Control group (group A) comprised healthy saddle horses (mean ± SD, 525.6 ± 46.9 kg; 6.8 ± 1.3 years old) with no history of respiratory tract disease that were unaffected by exposure to moldy hay and straw. The other group (group B; 567.0 ± 14.7 kg; 14.2 ± 3.6 years old) comprised saddle horses with COPD. Group-A horses were housed in stables for several weeks before scintigraphy was performed. Group-B horses were studied under 3 environmental conditions; after 2 months at pasture (remission 1); then, after 2 months in a well-ventilated barn with wood shavings or quality straw bedding and consumption of grass silage[a] (remission 2); and finally, in acute exacerbation of disease after natural challenge (crisis). The time to induce signs of COPD varied among horses (from 4 hours to 4 days). Scintigraphy was performed at least 1 day after appearance of signs of COPD.

Pulmonary function tests—Prior to scintigraphy, physical examination, respiratory tract endoscopy, and routine PFT (ie, measurement of **respiratory rate [RR]**, **total respiratory resistance [R_L]**, **dynamic lung compliance [C_{dyn}]**, **maximal change in pleural pressure [MaxΔPpl]**, **tidal volume [V_T]**, and arterial blood gas [Pa_{O_2} and Pa_{CO_2}] tensions on blood samples obtained by puncture of the carotid artery) were performed.

Mechanics of breathing were measured as follows. Pleural pressure was measured by use of a balloon sealed over the end of a semi-rigid catheter[b] that was positioned in the distal third of the esophagus via a nostril. The catheter was connected to a pressure transducer[c] calibrated with a water manometer. Horses were fitted with an airtight face mask, and respiratory airflow was measured using a heated Fleisch No. 4 pneumotachograph placed in an opening of the face mask. A pulmonary function computer[d] integrated the flow signal to determine V_T, and calculate C_{dyn} and R_L from measurements of pleural pressure, respiratory airflow, and V_T. Horses with COPD were considered in crisis when they had values for $R_L \geq 0.10$ kPa/L/s, MaxΔPpl > 1.75 kPa, and $Pa_{O_2} < 85$ mm Hg.

Radioactive ^{99m}Tc-DTPA aerosol preparation and generation—The ^{99m}Tc-DTPA was prepared by combining sodium pertechnetate ($Na^+[^{99m}TcO_4^-]$) with DTPA in a lyophilization reaction vial,[e] and diluting this solution with saline (0.9% NaCl) solution to obtain 6 ml of solution for nebulization. A pair of disposable jet nebulizers[f] was used to generate the aerosol. Particle size characteristics of the aerosol produced were determined, using a particle size analyzer.[g] The **mass median aerodynamic diameter (MMAD)** was found to be 1.2 μm with a **geometric standard deviation (GSD)** of 2.44 μm; > 80% of the aerosol mass consisted of particles with MMAD < 3 μm.

Inhalation procedure and image acquisition—Radioactive aerosol (7.4 MBq/kg of body weight) was delivered to sedated horses (0.04 mg of romifidine[h]/kg, IV) through an apparatus especially developed for radioactive aerosol administration in horses.[30] After 6 minutes of nebulization, input was discontinued, but the horse remained connected to the administration assembly for a few breaths so that undeposited ^{99m}Tc-DTPA aerosol particles could be collected. Horses then stood for 20 minutes with the left side of the thorax pressed against the face of a gamma camera,[i] and sequential images, 30 seconds in duration, of the caudal portion of the lung were taken.[j] Then, ^{99m}Tc-macroaggregates of human serum albumin[k] (0.15 MBq/kg) were administered IV, and images of the cranial and caudal aspects of the left lung were recorded. These images were connected by the computer to represent perfusion of the left lung.

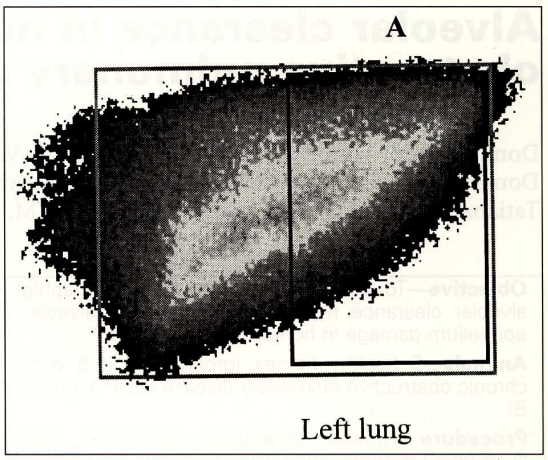

Figure 1—Scintigraphic scan from a horse of group A. Border of the scintigraphic perfusion image was used to delineate the ^{99m}Tc-DTPA deposition scan (inset) and to select the caudal half (A) of the left lung where clearance was studied.

Image processing and alveolar clearance kinetics—The edge of the perfusion image, defined by a line that visually fit the lung border, was used to outline the lung boundary of the ^{99m}Tc-DTPA deposition scans[31] and to delineate a region of interest for determining clearance rate. This region consisted of the caudal half of the left lung (**Fig 1**). The number of radioactive disintegrations (counts) recorded by the gamma camera in the region for each ^{99m}Tc-DTPA scintiscan was corrected for radioactive decay, using end of nebulization as time 0 (T_0). Activity versus time curves were generated from regional counts of each image. Correction for blood and tissue background activities was not performed.[32] Coefficient of time estimated by fitting monoexponential decay curves to the observed counts measured regional **clearance rate (k)**. Results also were expressed in half-time clearance from lung to blood ($T_{½} = \ln_2/k$).

Methacholine challenge experiments—Two days after scintigraphy (ie, when ^{99m}Tc radioactive decay was sufficient to avoid staff contamination), all horses were challenged with MCh chloride[l] administered by nebulization. The aerosol was produced by an ultrasonic nebulizer[m] that generated particles with MMAD of 1.3 μm and GSD of 1.91. Once again, particles with MMAD < 3 μm constituted > 80% of the aerosol mass emitted.

After control inhalation of saline solution, increasing doses of MCh (0.001, 0.01, 0.1, 1.0, 3.0, and 10 mg/ml) prepared on the test day were inhaled by tidal breathing for 2 minutes. After each inhalation procedure, mechanics of breathing were recorded for approximately 4 minutes. Serial challenges with MCh were repeated at 6-minute intervals. Response was measured by determining the concentration (mg/ml) of inhaled MCh that induced a **35% decrease in C_{dyn} from its baseline value ($PC_{35}C_{dyn}$)**. Sedation was not used during the challenges. Further details concerning the procedure are described elsewhere.[10]

Collection of bronchoalveolar lavage fluid

samples—Broncho-provocation challenge tests modify lung permeability,[33] which might induce modification in BAL cell populations. Therefore, BAL should not be performed after MCh inhalation. It has also been documented that BAL induces a localized pulmonary neutrophil influx.[34] Because it has been suggested that hyperresponsiveness is related to damage to, or inflammation of, airway epithelium,[35-37] this slight neutrophil infiltration might affect airway response to MCh. Consequently, collection of BAL fluid samples should not precede MCh challenge testing. It was decided to perform BAL after completion of scintigraphic and MCh challenge procedures during the various periods. Therefore, horses were again submitted to environmental conditions for the same time intervals as those previously described, and BAL then was performed. Before BAL fluid sample collection, it was verified that horses had similar PFT results as those obtained during the principal studies.

An endoscope[n] (diameter, 0.9 cm; length, 260 cm) was wedged in a bronchus and four 50-ml aliquots of saline solution (37 C) were successively instilled and withdrawn. The aspirated sample, preserved in an equal volume of 10% formalin, was used for determination of cell populations in the small airways and alveoli.

Statistics—A F-test was used to test differences in clearance rate within and between groups. Results of PFT and MCh challenge tests and cell populations in BAL fluid were evaluated, using two-sided unpaired and paired *t*-tests for comparisons between and within groups, respectively. Values of $P < 0.05$ were considered to be significant.

Results

Pulmonary function test results did not reveal significant differences between values for group-A horses and remissions 1 and 2 in group-B horses (**Table 1**). During COPD crisis, Pa_{O_2} and C_{dyn} decreased significantly, whereas RR, R_L, and MaxΔPpl increased significantly, compared with values for group-A horses and remissions 1 and 2 in group-B horses.

Mean (± SD) k and the corresponding $T_{½}$ values, as well as mean MCh concentration to induce $PC_{35}C_{dyn}$, were determined (**Table 2**). For remission 1, mean k in group-B horses was 1.98 ± 0.55%/min ($T_{½}$ = 37.73 ± 12.94 min), which did not differ significantly from mean value (k = 1.95 ± 0.33%/min; $T_{½}$ = 36.37 ± 5.85 min) in group-A horses. During crisis, ^{99m}Tc-DTPA clearance was significantly increased; mean k increased to 4.30 ± 0.95%/min ($T_{½}$ = 16.81 ± 3.86 min). After 2 months in a controlled environment, an intermediate value for mean k (3.20 ± 0.72%/min; $T_{½}$ = 22.50 ± 4.79 min) was observed.

The $PC_{35}C_{dyn}$ values for each horse were derived from the dose-response curve obtained after each MCh challenge test. During remission 1, mean bronchial reactivity was similar between the 2 groups. During crisis, group-B horses had hyperresponsive airways, and the $PC_{35}C_{dyn}$ values were significantly lower than values for remission 1 and group-A horses. During remission 2, $PC_{35}C_{dyn}$ values were significantly higher than those during crisis but were significantly lower than values for remission 1 and group-A horses. Results of the MCh challenge test confirmed mild hyperactivity during remission 2.

Macrophage and lymphocytes predominated in BAL cell populations, except for group-B horses during

Table 1—Mean (± SD) results of pulmonary function tests in control horses (group A, n = 5) and horses with chronic obstructive pulmonary disease (COPD; group B, n = 5) at pasture (remission 1), stabled in a controlled environment (remission 2), and during exacerbation of the disease induced by natural challenge (crisis)

		Group B		
Variable	**Group A**	**Remission 1**	**Remission 2**	**Crisis**
RR (breaths/min)	12.8 ± 3.0[a]	8.3 ± 1.5[a]	9.4 ± 4.0[a]	17.2 ± 7.2[b]
R_L (kPa/L/s)	0.05 ± 0.01[a]	0.07 ± 0.02[a]	0.06 ± 0.01[a]	0.34 ± 0.08[b]
C_{dyn} (L/kPa)	18.0 ± 2.3[a]	19.4 ± 4.4[a]	15.9 ± 2.9[a]	3.5 ± 2.0[b]
V_T (L)	4.3 ± 0.5	7.3 ± 2.5	6.7 ± 1.8	5.1 ± 2.2
MaxΔPpl (kPa)	< 0.8[a]	0.6 ± 0.1[a]	0.7 ± 0.1[a]	3.3 ± 0.7[b]
Pa_{O_2} (mm Hg)	105.9 ± 3.2[a]	109.7 ± 13.4[a]	98.1 ± 11.1[a]	75.1 ± 7.8[b]
Pa_{CO_2} (mm Hg)	43.7 ± 0.4	44.0 ± 2.8	45.1 ± 3.1	45.9 ± 2.5

[a,b]Within a row, means with different superscript letters are significantly ($P < 0.05$) different.
RR = respiratory rate. R_L = total respiratory resistance. C_{dyn} = dynamic lung compliance. V_T = tidal volume. MaxΔPpl = maximal change in pleural pressure.

Table 2—Mean (± SD) alveolar clearance rates (k) and the corresponding $T_{½}$ values and results of methacholine challenge tests (n = 5 for each group) in horses of groups A and B

		Group B		
Variable	**Group A**	**Remission 1**	**Remission 2**	**Crisis**
k (%/min^{-1})	1.95 ± 0.33[a]	1.98 ± 0.55[a]	3.20 ± 0.72[b]	4.30 ± 0.95[c]
$T_{1/2}$ (min^{-1})	36.37 ± 5.85[a]	37.73 ± 12.94[a]	22.50 ± 4.79[b]	16.81 ± 3.86[c]
$PC_{35}C_{dyn}$ (mg/ml)	5.08 ± 2.04[a]	3.81 ± 2.2[a]	1.48 ± 0.50[b]	0.23 ± 0.21[c]

[a,b,c]Within a row, values with different superscript letters are significantly ($P < 0.05$) different.
$PC_{35}C_{dyn}$ = Methacholine concentration (mean ± SD) necessary to induce a 35% decrease in dynamic lung compliance from its baseline value in the same groups of horses.
See Table 1 for key.

Table 3—Cell populations in bronchoalveolar fluid from horses (n = 5 for group A and n = 4 for group B*)

Cell type	Group A	Group B		
		Remission 1	Remission 2	Crisis
Macrophages (%)	64.9 ± 14.2[a]	38.0 ± 24.5[ab]	37.7 ± 7.8[b]	23.3 ± 9.4[b]
Lymphocytes (%)	27.7 ± 13.3[a]	55.0 ± 20.1[ab]	49.7 ± 6.8[b]	18.0 ± 8.14[a]
Neutrophils (%)	6.5 ± 3.8[a]	6.8 ± 7.8[a]	12.0 ± 9.9[a]	56.3 ± 16.3[b]
Eosinophils (%)	0.7 ± 0.8	0.0 ± 0.0	0.3 ± 0.5	1.3 ± 1.9

*Data from 1 horse were rejected because of clinical signs of infectious pulmonary disease.
See Table 1 for key.

crisis, in which neutrophils represented a majority (**Table 3**).

Discussion

Sedation is a prerequisite for gamma camera imaging.[31] However, in vitro and in vivo studies indicate that α_2-adrenergic agonists, through the medium of α_2-adrenergic receptors on equine airway cholinergic nerves,[38] affect pulmonary function of horses with COPD.[39,o,p] Administration of α_2-adrenergic agonists during exacerbation of the disease significantly decreases R_L and increases C_{dyn}. These functional modifications favor deeper aerosol penetration within airways. Alveolar absorption, bronchial absorption, and mucociliary clearance are the 3 main clearance mechanisms involved in ^{99m}Tc-DTPA disappearance. The proportion of aerosol deposited in various parts of lungs may influence regional clearance of ^{99m}Tc-DTPA.[40] Nevertheless, when half-time clearance calculations are performed on 20-minute clearance curves, influence of the slowest bronchial absorption and mucociliary clearance on the calculated values seems minimal.[1] Conversely, during COPD crisis, sedation favors peripheral deposition and therefore, more accurate measurement of alveolar clearance. Sedation does not seem to be a major drawback for alveolar clearance measurement in horses with COPD.

A major challenge encountered when studying the link between a potentially damaging stimulus (eg, aeroallergens) and pulmonary response is to assess the resulting pulmonary alteration. With mechanics of breathing and blood gas analysis, the pulmonary response must be marked to be measured,[q,r] and minimal lung injury, which does not cause an important modification of function, may go unnoticed. Studies of human beings indicate that an advantage of the ^{99m}Tc-DTPA test is possible detection of subclinical pulmonary involvement.[28,29]

Analysis of results of clinical examination, PFT, and MCh challenge testing and determination of BAL fluid cell populations and alveolar clearance rate permit clinicians to easily differentiate clinical status between remission obtained at pasture and crisis in horses with COPD.[1,10,11,14,24] Results of the principal and additional experiments were in agreement with previous findings concerning scintigraphic alveolar clearance rate[1] and BAL fluid cell populations.[7,11]

Unlike MCh challenge testing, clinical examination and PFT may not differentiate remission obtained at pasture from that of horses with COPD stabled in a controlled environment.[10] To the authors' knowledge, subclinical inflammatory processes have not been studied by means of scintigraphy or BAL fluid analysis.

The obvious differentiation of subgroups within group B by MCh challenge testing and ^{99m}Tc-DTPA clearance suggests that both techniques are sensitive for highlighting subclinical pulmonary damage in horses with COPD, despite lack of functional abnormalities. This is important, because severity of COPD is presumed to vary depending on environmental conditions and, more precisely, the balance between aeroallergen or airbone irritant concentration and lung responsiveness; lesser amounts of irritants may induce bronchospasm in housed horses that have hyperresponsive airways, compared with horses in remission at pasture.[10]

During the additional experiments, mechanics of breathing and arterial blood gas tensions were similar to those obtained during the principal experiments. These additional experiments were aimed at assessing whether the more easily performed and less expensive test (ie, determination of BAL cell populations) might underline subclinical inflammatory processes as accurately as scintigraphy. Obviously, there was an important overlap between mean percentage of neutrophils in horses without clinical signs of COPD or PFT modifications (ie, group A, group B in remissions 1 and 2), and determination of BAL fluid cell populations failed to indicate any subclinical inflammatory process in group-B horses when they were stabled in a controlled environment. Nevertheless, this conclusion must be recognized when considering that determination of BAL fluid cell populations was performed at another time and that despite the fact horses were exposed to identical environmental conditions, the subclinical status in remission 2 may have differed.

Detection of an inflammatory process in its early or subclinical stage is important because it permits treatment of alveolar injury before development of clinical signs of disease or irreversible structural deterioration, or both. The ^{99m}Tc-DTPA permeability study does not offer the opportunity to diagnose a specific disease, but enables quantification of lung damage. This quantitative information about alveolar-capillary barrier integrity may be used to follow the time course and severity of lung injury and to monitor the subsequent repair process (eg, after equine influenza, equine herpesvirus infection, or bronchopneumonia). A potential advantage of scintigraphy over agonist challenge testing is lack of lung response to the chemically inert and electrically neutral ^{99m}Tc-DTPA. Lack of interference with the system studied is a valuable quality for any investigative tool.

With regard to COPD, this technique enables detection of subclinical damage associated with stabling despite hygienic measures taken with regard to the environment. Chronic inflammation in young performance horses might represent an early stage of COPD.[41] Adequate management of these horses would be critical in preventing development of COPD. Conversely, horses with COPD, through the medium of alveolar clearance rate determination, could be used as indicators of the quality of a specific controlled envi-

ronment to define the most appropriate conditions for management of such horses.

In this study, ^{99m}Tc-DTPA clearance was a more sensitive indicator of lung damage than PFT. This noninvasive technique may be used to detect early changes at the alveolar level in horses with COPD and should be considered an additional diagnostic tool for detection of pulmonary dysfunction.

[a]Préfané Liégeois, Mortier, Belgium.
[b]Teflon, Vel, Leuven, Belgium.
[c]Validyne MP-45, Validyne Engineering, Northridge, Calif.
[d]Hemodynamic Respiratory System, Medisoft, Dinant, Belgium.
[e]TechneScan, Mallinckrodt Medical, Petten, Holland.
[f]Neb-U-Mist 1730 Up-DraftII JN, Hudson RCI, Temecula, Calif.
[g]PCS2000, Palas, Karlsruhe, Germany.
[h]Sedivet, Boehringer Ingelheim, Brussels, Belgium.
[i]Maxicamera Gimbal, GE Medical systems, Milwaukee, Wis.
[j]Sophy, Sopha Medical, Paris, France.
[k]LyoMAA, Mallinckrodt Medical, Petten, Holland.
[l]Sigma Chemical Co, St Louis, Mo.
[m]DeVilbiss Ultra-Neb 200HI, Somerset, Pa.
[n]Pentax EPM-3000, Asahi Optical Co, Japan.
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[q]Robinson NE, Berney C, Olszewski M, et al. Determinants of maximal changes in pleural pressure in horses with heaves, in *Proceedings*. Annu Meet Comp Respir Soc 1994;13:A–11.
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