EQUINE

Effect of an external nasal dilator strip on cytologic characteristics of bronchoalveolar lavage fluid in Thoroughbred racehorses

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Objective—To determine the effects of an external nasal dilator strip on cytologic characteristics of bronchoalveolar lavage (BAL) fluid in racing Thoroughbreds.

Design—Clinical trial.

Animals—23 Thoroughbred racehorses in active training.

Procedure—Each horse raced on 2 occasions: once while wearing an external nasal dilator strip and once while not. Bronchoalveolar lavage was performed 12 to 18 hours after each race, and BAL fluid was analyzed for RBC and leukocyte counts and hemosiderin content.

Results—Mean ± SEM count of RBCs in BAL fluid when horses raced without the nasal dilator strip (84.6 ± 27.5 cells/µL) was not significantly different from count when they raced with it (41.7 ± 12.2 cells/µL). Horses were grouped as having mild or severe bleeding on the basis of RBC count in BAL fluid after horses raced without the nasal dilator strip. Mean count when horses with severe bleeding raced without the nasal dilator strip (271.0 ± 63.7 cells/µL) was significantly higher than mean count when these horses raced with the strip (93.8 ± 37.6 cells/µL). Mean count of lymphocytes in BAL fluid was significantly lower after horses raced with the external nasal dilator strip.

Conclusions and Clinical Relevance—Results suggest that use of an external nasal dilator strip in Thoroughbred racehorses may decrease pulmonary bleeding, particularly in horses with severe exercise-induced pulmonary hemorrhage. (*J Am Vet Med Assoc* 2004;224:558–561)

Exercise-induced pulmonary hemorrhage (EIPH) in horses is defined as bleeding from the lungs as a consequence of exercise.[1] In previous studies,[1,2] the incidence of EIPH in horses was determined to be 44% by means of endoscopy of the trachea and 75% by means of endoscopy of the tracheal bifurcation. Endoscopic findings in individual horses with EIPH are repeatable, suggesting that bleeding is not a random event.[2] Areas affected by EIPH have been reported to be bilateral, focal, and located in the caudodorsal lung regions.[3] **Bronchoalveolar lavage (BAL)** has been determined to be a useful technique for diagnosis of EIPH. The catheter used to perform BAL usually wedges in a subsegmental bronchus of the left or right caudodorsal lung region.[4,5]

Bronchoalveolar lavage has been used to diagnose EIPH[6,7] and provides a more sensitive and accurate assessment for the presence and extent of hemorrhage than does endoscopy of the trachea.[6] The prevalence of EIPH in exercising horses has been determined by means of BAL to be at least 90%. In addition, cytologic evaluation has demonstrated that most, if not all, horses in race training experience EIPH after high-intensity exercise.[8]

In humans, an external nasal dilator strip has been used to modify nasal airflow resistance in a variety of conditions associated with symptoms of nasal obstruction.[9] In horses, an external nasal dilator strip has been used to prevent or reduce collapse of the nasal passages, decrease upper airway resistance, and reduce changes in intrapleural and alveolar pressures that may contribute to high pulmonary capillary transmural pressures and EIPH.[10] Recent studies[10-12] have shown a 20% to 40% reduction in the RBC count of BAL fluid with the use of an external nasal dilator strip. However, these studies were performed in horses exercising on a high-speed treadmill, and there are differences[13] in response to exercise between horses running on the track and on a treadmill. To our knowledge, the effect of nasal dilator strips in horses that are actively racing has not been determined. The purpose of the study reported here was to determine the effects of an external nasal dilator strip on cytologic characteristics of BAL fluid in racing Thoroughbreds.

Materials and Methods

Study design—The study was performed at Golden Gate Fields Racetrack in California between November 8, 2001, and March 28, 2002. Thirty Thoroughbred horses in active racing with a history of EIPH were selected for participation, of which 23 completed the study. Age of the 23 horses that completed the study ranged from 2 to 4 years (mean, 3.04 years). There were 8 fillies and 15 males. All horses from 3 trainers were included in the study with the exception of 1 horse that was excluded at the owner's request.

Owners of horses included in the study agreed to race the horses on 2 occasions—once while the horse was wearing an external nasal dilator strip[a] and once while it was not—and to have BAL performed between 12 and 18 hours after racing. Mean interval between races was 26 days. Horses that were claimed or raced only once because of a musculoskeletal lesion or for which the difference in distance between races was > 1 furlong were excluded from the study.

Bronchoalveolar lavage technique—All horses were sedated with 250 mg of xylazine. Debris was cleaned from the nostrils with moist gauze sponges. A 3-m-long BAL catheter[b]

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was introduced into the left nostril and advanced into the trachea until it lodged in 1 of the terminal bronchi. While the catheter was advanced, 60 mL of 1% lidocaine was infused into the trachea in an effort to desensitize it and decrease the incidence of coughing. Once the BAL catheter was lodged in place, the balloon at the distal end of the catheter was insufflated with 5 mL of air. One hundred milliliters of sterile saline (0.9% NaCl) solution was instilled and then withdrawn with a syringe; low pressure was used to minimize trauma. When 50% of the infused fluid was recovered, aspiration was stopped and the sample obtained was stored in plastic tubes on ice.

All BAL fluid samples were analyzed within 2 hours after collection. Because the clinician who performed BAL was in contact with the trainers and the track veterinarian, it was not possible to perform BALs in a blinded manner. However, samples were analyzed in a blinded fashion by 1 of the investigators (SDO). Red blood cell counts were performed manually with a hemocytometer; leukocyte counts were performed with an automated system.[c] Two slides were prepared from each sample with a cytocentrifuge.[d] One was stained with Wright-Giemsa stain for differential leukocyte counting. The other was stained with Prussian blue to determine hemosiderin content of macrophages, and a hemosiderin score was calculated as described.[14] In brief, the investigator examined 200 macrophages and graded hemosiderin content in each macrophage from 0 to 3. Grade 0 indicated that no stain was retained. Grade 1 indicated that a minimal amount of stain, identified as slight, diffuse, green-black to brown-black stain or a few clumps of green-black to brown-black stain involving < 25% of the cytoplasm, was seen. Grade 2 indicated that a moderate amount of stain, identified as diffuse, green-black to brown-black stain or some clumps of green-black to brown-black stain involving 25% to 50% of the cytoplasm, was seen. Grade 3 indicated that a marked amount of stain, identified as diffuse, green-black to brown-black stain or many large clumps of green-black to brown-black stain involving 50% to 100% of the cytoplasm, was seen. The hemosiderin score was then calculated by means of the following equation: ([1 × number of grade-1 macrophages] + [2 × number of grade-2 macrophages] + [3 × number of grade-3 macrophages])/2. The maximum possible score was 300.

Data analysis—A **performance index (PI)** was calculated for the 2 races in which each horse raced by use of an adaptation of a previously published formula.[15] Briefly, points were assigned on the basis of finishing position in each race (first place, 5 points; second place, 4 points; third place, 3 points; fourth place, 2 points; and not placed, 1 point). A scalar value was then assigned on the basis of the purse for that race (≥ $15,000, 1; $10,000 to $14,999, 0.8; $5,000 to $9,999, 0.6; and < $5,000, 0.4). The PI was then calculated by multiplying the placement points by the scalar value for each race and dividing the value for the race when the horse wore the nasal dilator strip by the value for the race when the horse did not wear the nasal dilator strip. Horses that had a PI > 1 were considered to have performed better when wearing the nasal dilator strip, and horses that had a PI < 1 were considered to have performed worse when wearing the nasal dilator strip. In horses in which the PI = 1, the nasal dilator strip was considered to have had no effect on performance.

Results of analyses of BAL fluid obtained after racing with and without the external nasal dilator strip were compared by means of paired Student *t* tests. A value of $P < 0.05$ was considered significant.

Results

Seven of the original 30 horses selected for inclusion in the study were excluded because they were claimed or retired owing to a musculoskeletal lesion or because the difference in distance between the 2 races was > 1 furlong. Of the 23 horses that completed the study, 15 raced first without the external nasal dilator strip and 8 raced first with the external nasal dilator strip. In 2 horses, RBC counts in BAL fluid collected after 1 of the races were not measured within 2 hours after sample collection and results of BAL fluid analyses were not included for these horses. All horses received a single dose of furosemide (0.05 mg/kg [0.023 mg/lb], IV) 4 hours before each race. The dose was calculated on the basis of body weight estimated by the track veterinarian.

Mean ± SEM count of RBCs in BAL fluid was not significantly (P = 0.054) higher when horses raced without the nasal dilator strip (84.6 ± 27.5 cells/µL) than when they raced with it (41.7 ± 12.2 cells/µL). Horses were sorted on the basis of RBC count in BAL fluid when racing without the nasal dilator strip and grouped as having mild (n = 16) or severe (5) bleeding by dividing at the 75th percentile. In horses classified as having only mild bleeding, mean ± SEM count of RBCs in BAL fluid when horses raced without the nasal dilator strip (26.3 ± 6.0 cells/mL) was not significantly different (P = 0.901) from mean count when horses raced with the strip (25.4 ± 8.4 cells/µL). In contrast, for horses classified as having severe bleeding, mean count when horses raced without the nasal dilator strip (271.0 ± 63.7 cells/µL) was significantly (P = 0.046) higher than mean count when horses raced with the strip (93.8 ± 37.6 cells/µL).

Mean total leukocyte count when horses raced with the nasal dilator strip (378 ± 104 cells/µL) was not significantly (P = 0.43) different from mean count when they raced without the strip (513 ± 134 cells/µL). When horses were grouped as having mild or severe bleeding, there was still no significant difference between treatments. Mean hemosiderin score when horses raced without the nasal dilator strip (41 ± 10) was not significantly (P = 0.35) different from score when they did (52 ± 13). There were no significant differences in absolute or differential counts of macrophages, neutrophils, eosinophils, and mast cells in BAL fluid when horses raced with versus without the nasal dilator strip (**Table 1**). However,

Table 1—Results of cytologic analysis of bronchoalveolar lavage fluid obtained from 23 Thoroughbred racehorses 12 to 18 hours after horses raced with or without application of an external nasal dilator strip

Variable	Without nasal strip	With nasal strip
Absolute count (cells/µL)		
Macrophages	257.2 ± 19.0	294.7 ± 16.7
Lymphocytes	231.2 ± 17.7*	162.4 ± 14.4
Neutrophils	27.8 ± 8.2	34.7 ± 10.0
Eosinophils	0.4 ± 0.2	4.2 ± 2.1
Mast cells	2.4 ± 0.9	4.00 ± 1.2
Differential count (%)		
Macrophages	51.3 ± 3.8	58.9 ± 3.4
Lymphocytes	42.7 ± 3.5*	32.5 ± 2.9
Neutrophils	5.6 ± 1.6	6.9 ± 2.0
Eosinophils	0.07 ± 0.03	0.8 ± 0.4
Mast cells	0.5 ± 0.2	0.8 ± 0.2

Data are given as mean ± SEM.
*Significantly ($P < 0.05$) different from value obtained when horses raced with the nasal dilator strip.

mean absolute and differential lymphocyte counts when horses raced without the nasal strip were significantly (P = 0.04) higher than counts when horses raced with the nasal strip.

Performance index was calculated for all 23 horses that completed the study. Six (26%) had an increase in performance, 10 (44%) had no change in performance, and 7 (30%) had a decrease in performance when wearing the nasal dilator strip versus when not wearing the strip.

Discussion

During BAL, the catheter usually lodges in 1 of the terminal bronchi of the caudodorsal lung region,[4,5] which is the area of lung that has been implicated in EIPH.[3,16] Although we could not control where BAL catheters used in the present study lodged, a previous study[7] found no difference between numbers of RBCs, leukocytes, and hemosiderophages in lavage fluid from the right and left lungs. Factors that can influence the number of cells recovered in BAL fluid include the volume of fluid infused, the volume of fluid recovered, and the pressure used to withdraw the lavage fluid.[17] In the present study, we attempted to at least partially control for these variables by injecting and collecting the same volumes of fluid in all horses. Participants at the International Workshop on Equine Chronic Airway Disease have recommended using a lavage volume of 250 to 500 mL.[18] We decided to use a smaller volume (100 mL) because of concerns expressed by the track veterinarian and trainers. All horses used in the present study were privately owned and in active training and racing. Because use of smaller volumes may result in lavage only of the bronchioles, and bronchioles tend to have a greater proportion of neutrophils and higher total leukocyte counts than do the alveoli,[17] use of small lavage volumes for BAL is likely to yield differential cell counts different from those expected with the use of larger volumes, which result in lavage of the bronchioles and alveoli. Dilution may also be a problem in interpreting results of BAL, but no standardized method of adjusting results of BAL fluid analysis for dilution has been determined.[17] In the present study, we decided to discontinue aspiration of fluid after 50% of the infused fluid was recovered because this was within the usual range reported for the proportion of lavage fluid typically recovered (40% to 60%).[18]

Iatrogenic hemorrhage resulting from trauma caused by a BAL catheter or barotrauma during aspiration of fluid can also affect the results of BAL fluid analysis.[19] In the present study, care was taken to avoid overinflation of the catheter's balloon and the fluid was carefully withdrawn manually, although aspiration by hand may yield a lower volume of fluid than use of a vacuum pump.[20] Although we did not use a pump to maintain a constant suction pressure, care was taken to avoid excessive negative pressure. In addition, all BALs were performed by a single individual (SCV) to decrease variations that could have influenced the results.

Exercise-induced pulmonary hemorrhage apparently results from stress failure of pulmonary capillaries caused by high transmural pulmonary capillary pressure across the alveolus.[16] Most (40% to 50%) of the total pulmonary resistance during exercise is created by the nasal passages.[21] External nasal dilator strips such as the ones used in the present study tent the skin over the nasal valve, which dilates the nasal passages and decreases airway resistance,[22] thus potentially decreasing the severity of pulmonary hemorrhage.[10,12]

Recent studies[10-12] found that RBC counts in BAL fluid from horses running on a treadmill decreased 20% to 40% when an external nasal dilator strip was applied. However, in these studies, horses were exercised on a high-speed treadmill. There are reported differences in the response to exercise between horses running on a track and on a treadmill.[13] These differences could be attributed to effects of the rider, a lack of speed control while running on a track, the biomechanics of treadmill locomotion, variations in airstream and atmospheric conditions, and extent of psychological adaptation to a treadmill situation. In contrast, the present study was performed with Thoroughbreds that were actually racing to more closer replicate the real-life conditions under which nasal dilator strips would be used.

In the present study, when all horses were considered, there was no significant difference in RBC counts in BAL fluid obtained after horses raced with versus without a nasal dilator strip, although the P value was close to the cutoff for significance (P = 0.054). Because the power of this comparison was low (0.49), we decided to explore the possibility that nasal strips may have had a more appreciable effect in horses that had more severe EIPH. When horses were classified as having mild or severe bleeding, we observed a significant reduction in the RBC count of BAL fluid from horses classified as having severe bleeding when wearing the nasal dilator strip, although there was no significant difference in horses classified as having mild bleeding. Thus, our results suggest that horses with more substantial hemorrhage may obtain more benefit from use of nasal dilator strips than mildly affected horses.

Several studies[6,23] involving analysis of BAL fluid from clinically normal horses have been published, and results vary considerably, making comparison of results between studies difficult. Most of these differences are likely attributable to variations in BAL technique.[17] In general, however, there is an increase in the number of RBCs in BAL fluid 90 minutes after exercise, with RBC counts decreasing to pre-exercise values approximately 1 week after exercise.[7] After inoculation of autologous blood into the lungs of horses, the mean RBC count in the BAL fluid does not change significantly for 5 days.[24] In the present study, BAL was performed 12 to 18 hours after racing. Therefore, values obtained would be expected to be similar to those obtained if BAL had been performed immediately after exercise.

Small numbers of RBCs and hemosiderophages can be found in BAL fluid of resting horses.[24] Therefore, to determine when hemorrhage has occurred, standardized methods for semiquantitative assessment of hemosiderophages in BAL fluid need to be adopted.[25] In the present study, hemosiderin scores when horses raced with the nasal dilator strip were not

significantly different from scores when horses raced without the strip. All Thoroughbred racehorses have some hemosiderin-laden macrophages in tracheal wash fluid as a result of training,[26] and hemosiderophages in BAL fluid may be a result of recent or past hemorrhage.[8,24] In the present study, we had no control over the racing and training schedules of the horses, and some of the hemosiderophages observed in the BAL fluid could have been a result of previous hemorrhage.

Reference values for total leukocyte count in BAL fluid vary with technique and volume of fluid infused and retrieved.[17] Most cells are macrophages and lymphocytes,[6,24] with the remainder being nondegenerative neutrophils, mast cells, epithelial cells, and eosinophils.[6,8,17] In horses with mild hemorrhage not detectable by means of endoscopy, the leukocyte count in BAL fluid does not increase,[7] suggesting that leukocytes are not recruited to the alveolar space by the entry of RBCs. In our study, although there was no significant difference in total leukocyte counts in BAL fluid when horses raced with or without the external nasal dilator strip, the absolute lymphocyte count and the proportion of cells that were lymphocytes in BAL fluid were significantly lower when horses raced with the nasal dilator strip than when they did not. We were not able to determine why this difference was seen. However, a previous study[14] showed that in horses with EIPH, exercise resulted in an increased total nucleated cell count, lymphocyte count, and percentage of lymphocytes in BAL fluid. These changes were thought to be secondary to increased mobilization of cells from lung tissue or an influx of inflammatory cells into the lungs. Another study[27] found a significantly higher eosinophil count in BAL fluid from horses with EIPH, compared with control horses.

In the present study, we calculated a PI to determine whether the nasal dilator strip had an effect on overall race performance of the horses and did not find any consistent improvement in performance with the use of the nasal strip. In conclusion, therefore, results of the present study suggest that use of an external nasal dilator strip in Thoroughbred racehorses may decrease pulmonary bleeding, particularly in horses with severe EIPH, but likely will not have any appreciable effect on overall race performance.

[a]Flair nasal strip, CNS Inc, Minneapolis, Minn.
[b]Bivona nasotracheal tube, Bivona Inc, Gary, Ind.
[c]Baker 9000, Serono-Baker Diagnostics Inc, Allentown, Pa.
[d]Cytospin 2, Shandon Inc, Pittsburgh, Pa.

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