Efficacy of nasal strip and furosemide in mitigating EIPH in Thoroughbred horses

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Kindig, Casey A., Paul McDonough, Gus Fenton, David C. Poole, and Howard H. Erickson. Efficacy of nasal strip and furosemide in mitigating EIPH in Thoroughbred horses. J Appl Physiol 91: 1396–1400, 2001.—The purpose of this investigation was to study the effects of an equine nasal strip (NS), furosemide (Fur), and a combination of both (NS + Fur) on exercise-induced pulmonary hemorrhage (EIPH) at speeds corresponding to near-maximal effort. Five Thoroughbreds (526 ± 25 kg) were run on a flat treadmill from 7 to 14 m/s in 1 m·s⁻¹·min⁻¹ increments every 2 wk (treatment order randomized) under control (Con), Fur (1 mg/kg iv 4 h prior), NS, or NS + Fur conditions. During each run, pulmonary arterial (Ppa) and esophageal (Pes) pressures were measured. Severity of EIPH was quantified via bronchoalveolar lavage (BAL) 30 min postrun. Furosemide (Fur and NS + Fur trials) reduced peak Ppa ~7 mmHg compared with Con (P < 0.05) whereas NS had no effect (P > 0.05). Maximal Pes swings were not different among groups (P > 0.05). NS significantly diminished EIPH compared with the Con trial [Con, 55.0 ± 36.2; NS, 30.8 ± 21.8 × 10⁶ red blood cells (RBC)/ml BAL fluid; P < 0.05]. Fur reduced EIPH to a greater extent than NS (5.2 ± 3.0 × 10⁶ RBC/ml BAL; P < 0.05 vs. Con and NS) with no additional benefit from NS + Fur (8.5 ± 4.2 × 10⁶ RBC/ml BAL; P > 0.05 vs. Fur, P < 0.05 vs. Con and NS). In conclusion, although both modalities (NS and Fur) were successful in mitigating EIPH, neither abolished EIPH fully as evaluated via BAL. Fur was more effective than NS in constraining the severity of EIPH. The simultaneous use of both interventions appears to offer no further gain with respect to reducing EIPH.

bronchoalveolar lavage; pulmonary capillary stress failure; pulmonary arterial pressure; exercise-induced pulmonary hemorrhage

EXERCISE-INDUCED PULMONARY HEMORRHAGE (EIPH) is a condition afflicting nearly all racehorses during high-intensity running. The severity of EIPH ranges from negligible blood in the lower airways detectable only via bronchoalveolar lavage (BAL) to frank epistaxis (14, 18, 20, 35). Although minimal EIPH has been reported in humans after intense cycling (13), the severity of EIPH in the Thoroughbred horse is a unique phenomenon. The Thoroughbred is sui generis in that it generates immense cardiac outputs and ventilatory volumes during exercise such that extremely positive intravascular and negative extravascular pressures are incurred within the pleural cavity. These pressures summate at the fragile blood-gas barrier, resulting in capillary stress failure and rupture-induced extravasation of red blood cells (RBC) into the alveoli (6, 26, 32, 33).

EIPH is a serious medical concern in that it increases recovery time between heavy bouts of running, likely impairs performance (34), and tends to worsen with repeated exercise and increased age (17, 18). Furthermore, severe EIPH may result in missed races, track banishment, and, in extreme cases, acute mortality (9, 35). Therefore, the development of effective prophylaxis for EIPH has been sought for over 20 years. Present treatments are limited, in large part, to the use of furosemide (Fur), a high-ceiling loop diuretic, which reduces plasma volume (12) and pulmonary arterial pressure (Ppa) as well as pulmonary capillary wedge pressure (8, 15, 16, 22). Furthermore, Fur has bronchodilatory properties that have been demonstrated to reduce airway resistance in ponies and horses (1, 5). Nearly 75% of all Thoroughbreds are given Fur before races in the United States (10). Despite widespread use, its administration is controversial because of questioned efficacy in mitigating EIPH, ability to mask other drugs, and its performance enhancing properties (10). Recently, an equine nasal strip (NS) was developed to maintain upper airway patency and thus reduce airway resistance during inspiration. Our laboratory (25) has demonstrated that NS application resulted in a 33% mean reduction in EIPH during intense treadmill exercise with the greatest benefit afforded to the worst “bleeders.”

Endoscopic evidence at the racetrack has supported the role of Fur in reducing the severity of EIPH (24) although several studies have been unable to define clearly what role (if any) Fur plays in reducing the incidence and severity of EIPH (27, 29, 30). As far as we are aware, only one investigation has utilized BAL to examine the efficacy of Fur. Lester et al. (14) found
that Fur administered intravenously 30 min before running attenuated EIPH whereas administration 4 h prior did not. Possible confounding variables in that investigation include the following: 1) the horses ran on a track and not in a climate-controlled equine treadmill laboratory, thus introducing certain vicissitudinous environmental variables; and 2) BAL cytology indicated that the degree of hemorrhage was minimal compared with other published values (18, 20, 25).

The purpose of this investigation was to employ a randomized, repeated-measures experimental design to quantify the severity of EIPH under controlled, laboratory conditions. Thus five horses performed identical incremental exercise tests on a motorized treadmill under the following conditions: 1) control, 2) equine nasal strip (NS), 3) Fur (1 mg/kg iv, 4 h prior; consistent with racetrack regulations), and 4) a combination of both (NS + Fur) to test the following hypotheses. First, NS administration would attenuate EIPH as a result of reduced airway resistance (and thus reduced intrapleural pressure swings) as estimated via analysis of esophageal pressure (Pes; estimate of pleural pressure) swings. Second, Fur would reduce EIPH as a result of lower Ppa and possibly via reduced Pes swings.

METHODS

Five healthy Thoroughbred geldings (age 7 ± 1 yr) trained twice weekly were used in this study. The animals were housed in a dry lot with free access to water and were fed twice daily. Food and water were withheld 4 h before exercise testing. All procedures were approved by the Kansas State University Institutional Animal Care and Use Committee.

Animal preparation and data acquisition. Before each experiment, the horse had one 7-F introducer catheter placed in the right jugular vein under local anesthesia (2% lidocaine) using aseptic techniques. A 7-F microtip pressure transducer (Millar Instruments, model SPC-471A, Houston, TX) was advanced through the introducer catheter 8 cm past the pulmonic valve into the pulmonary artery as determined via pressure waveforms viewed on a monitor via a computer-based data acquisition system (DATAQ, Akron, OH). In addition, an air-filled esophageal pressure balloon catheter connected to a differential pressure transducer (Validyne, model MC1-3-871, Northridge, CA) was placed in the midthoracic region of the esophagus (~150 cm from the nares). The pressure transducers were calibrated before and after each run with a mercury manometer. No transducer drift was detected in any of the runs.

Experimental protocol. Each horse completed four identical runs in 2-wk intervals on a level equine treadmill (Sato, Uppsala, Sweden). Control (Con), NS (FLAIR, CNS, Minneapolis, MN), Fur (Fur, 1 mg/kg iv 4 h prior), and NS + Fur runs were completed in randomized order. The NS, which contains three springs, was placed 1.5 inches above the nostrils with a positioning guide. The springs in the strip provide reinforcement for the unsupported tissue on the sides of the nose, rostral to the nasoincephalic notch and between the nasal and incisive bones. The protocol consisted of 1) warm-up trot (3 m/s for 4 min), 2) an incremental exercise test (1 m/s 0.5 min⁻¹ from 7 to 14 m/s), and 3) cool-down trot (3 m/s for 4 min).

Bronchoalveolar lavage. Bronchoalveolar lavage was performed 30 min postexercise. Initially, the horses were tranquilized with detomidine (10–20 µg/kg iv) to facilitate the BAL and to quantify the severity of EIPH (20). A BAL tube (Bivona Medical Technology, Gary, IN) was passed through the right naris until wedged in a subsegmental bronchus of the dorsal caudal portion of the lung (19). Next, a total of 300 ml (in 50-ml aliquots) of lactated Ringer solution was infused. After two breaths, the fluid (a percentage of the entire 300 ml) was aspirated. Fluid recovery averaged 63%; no significant differences in recovery existed between trials. The BAL fluid was then centrifuged, the supernatant was decanted, and the pellet was resuspended in PBS. The amount of PBS ranged from 10 to 200 ml depending on the severity of EIPH to maintain a relatively similar RBC-to-PBS solution ratio (i.e., hematocrit) to normalize instrument precision between trials. RBC were counted by an automated cell counter (MWI-DANAM Electronics, model VET7, Dallas, TX). Data are presented as RBC per milliliter of recovered BAL fluid minus tube dead space.

Statistical analysis. All data are presented as means ± SE. Differences in Ppa and Pes between Con, NS, Fur, and NS + Fur were tested via a one-way ANOVA with repeated measures. Differences in EIPH were tested via one-way repeated-measures ANOVA on ranks. When significant differences were found, a Student-Newman-Keuls post hoc test was used to determine individual differences. Statistical significance was accepted at P < 0.05.

RESULTS

Body mass. The horses weighed ~533 kg 4 h before the exercise test; there were no differences (P > 0.05) across the four trials. Immediately after the Con and NS exercise tests, body weights were reduced an average of 16 ± 4 kg (P > 0.05 between Con and NS). Mean weight loss was 24 ± 3 kg after the Fur and NS + Fur (P > 0.05 between Fur and NS + Fur), which was a significantly greater (P < 0.05) reduction compared with the Con and NS runs.

Peak pulmonary arterial and esophageal pressures (in all instances obtained at 14 m/s). Fur and NS + Fur trials reduced Ppa ~7 mmHg (P < 0.05, β = 1.0) compared with Con (Fig. 1). No differences in Ppa compared between NS + Fur and Fur (P > 0.05). NS
administration alone did not alter Ppa \((P > 0.05; \text{Fig. 1})\). Maximal Pes (i.e., maximum Pes – minimum Pes) changes were similar \((P > 0.05, b = 0.05)\) between groups (Con, 96.2 ± 8.2; NS, 94.2 ± 8.9; Fur, 98.8 ± 6.6; NS + Fur, 105.0 ± 8.2 cmH₂O).

**EIPH.** Nasal strip application significantly diminished EIPH compared with the Con trial (Con, 55.0 ± 36.2; NS, 30.8 ± 21.8 × 10⁶ RBC/ml BAL fluid; \(P < 0.05; \text{Fig. 2A}\). Fur also reduced EIPH to 5.2 ± 3.0 × 10⁶ RBC/ml BAL fluid \((P < 0.05 \text{ vs. Con and NS})\). However, NS + Fur exhibited no additive effect \((8.5 ± 4.2 × 10⁶ \text{ RBC/ml BAL fluid; } P > 0.05 \text{ vs. Fur, } P < 0.05 \text{ vs. Con and NS; } \text{Fig. 2A}\). As shown in Fig. 2B, the horse that hemorrhaged the least during Con received no apparent benefit from either NS or Fur. Reconstituted blood in lactated Ringer depicting the mean response for each trial as well as recovered lavage fluid from a resting horse is shown in Fig. 3.

**DISCUSSION**

The principal original findings of this investigation, in horses run to 14 m/s \((-95\% \text{ maximal } O_2 \text{ uptake})\), are as follows. 1) Both NS and Fur reduced the severity of EIPH as assessed by bronchoalveolar lavage. However, Fur decreased EIPH to a significantly greater extent than NS \((\text{Fur, } -90\%; \text{NS, } -44\%)\), and the reduction of EIPH was not potentiated by using the NS in combination with Fur. 2) The Fur-induced decrease of EIPH was associated with a reduced mean pulmonary arterial pressure \((\text{Fur and Fur + NS trials})\) whereas NS was not. 3) In no condition were the peak swings in esophageal pressure altered.

**Esophageal pressure swings.** One putative mechanism by which the NS might reduce EIPH and also pulmonary \(O_2\) uptake during heavy exercise \((25)\) involves the effect of a reduction in inspiratory resistance on intrapleural pressure swings. Specifically, we hypothesized that the NS would reduce the esophageal pressure swings, reflecting the generation of less negative intrapleural pressures. In turn, this would reduce the contribution of negative intrapleural pressure to pulmonary capillary transmural pressure and thus decrease the opportunity for pulmonary capillary stress failure. However, neither NS nor NS + Fur conditions induced any alteration in the magnitude of the esophageal pressure swings. It is pertinent that indirect measurement of intrapleural pressure via an esophageal balloon reflects local changes in intrathoracic pressure \((2, 7)\), and thus the possibility that intrapleural pressures may have been altered by NS use in other regions of the lung cannot be discounted. The location of the esophageal balloon within the caudal thoracic esophagus places it within the same horizontal plane as the dorsal proximity of the lung \((\text{similar to the predominant site of EIPH; Refs. 17, 21})\), whereas the greatest intrapleural pressure swings are found in the middle and ventral thoracic planes \((7)\). It is worth noting that there were subtle differences in the esophageal waveform on the NS and NS + Fur trials compared with Con and Fur trials. Specifically, the esophageal pressure spikes associated with the footfalls of the forelimbs at 14 m/s often demonstrated a second spike at the end of inspiration that was more pronounced on.

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**Fig. 2.** Exercise-induced pulmonary hemorrhage (EIPH) was reduced significantly \((*P < 0.05)\) in the NS, Fur, and NS + Fur trials compared with Con. Fur and NS + Fur further lessened \((#P < 0.05)\) the EIPH response compared with NS administration; however, simultaneous NS and Fur use offered no further benefit \((A)\). Note that the horse that bled the least during the Con trial received no apparent benefit from NS or Fur \((B)\). Proportional change in EIPH calculated as \[\left\{-\frac{\text{EIPH}_{\text{Con}} - \text{EIPH}_{\text{NS}, \text{Fur, or NS + Fur}}}{\text{EIPH}_{\text{Con}}}ight\}\] BAL, bronchoalveolar lavage; RBC, red blood cells.

**Fig. 3.** Representative samples of lactated Ringer (blank) before infusion and from a lavage before any exercise \((\text{Rest})\). The 4 postexercise samples represent mean values \((\text{reconstituted blood in lactated Ringer})\) from the 4 trials: Con 55.0 ± 36.2; NS 30.8 ± 21.8; Fur 5.2 ± 3.0; NS + Fur 8.5 ± 4.2 × 10⁶ \text{RBC/ml BAL fluid.}
the NS runs. In addition, it appeared that the negative baseline was elevated in the NS run compared with Con; however, given variability between and within trials, these findings were not pursued further. Whether this reflects some alteration in lung volume or breathing pattern remains to be elucidated.

**Basis for Fur-induced reduction in EIPH.** In the present investigation, use of both the NS and Fur was effective in mitigating EIPH severity; however, in none of the runs was EIPH abolished (Figs. 2 and 3). These findings confirm those reported previously (25) in which EIPH was reduced via NS application, although, as discussed above, the similar maximal changes in esophageal pressure lend no additional insight into the precise mechanism for that reduction. A novel and more surprising finding was how efficacious Fur was in mitigating EIPH.

As discussed in the INTRODUCTION, the etiology of EIPH appears due most likely to very positive intravascular pressures combined with negative extravascular pressures, resulting in the mechanical failure of the blood-gas barrier (6, 26, 32, 33). Thus, if Fur reduces pulmonary vascular pressures as shown repeatedly by others (8, 15, 16, 22) and as evidenced in the present investigation, then the severity of EIPH should be reduced. Indeed, Fur lowered Ppa~7 mmHg and EIPH ~90%. It may seem inconceivable that such a relatively small reduction in Ppa from ~97 to ~90 mmHg resulted in such a large reduction in EIPH. However, a recent study by Birks et al. (4) may lend some insight regarding this issue. Specifically, Birks and colleagues studied the effects of altered transmural pressures from 25–150 mmHg in excised Thoroughbred lungs and quantified pulmonary capillary endothelial and epithelial breaks as a function of transmural pressure. Of specific interest, the number of endothelial breaks per millimeter of capillary did not increase significantly from 25 to 75 mmHg; however, a nearly fourfold increase in endothelial breaks occurred when transmural pressure was increased from 75 to 100 mmHg. In addition, the authors stated that, above 75 mmHg, the number of RBC in the alveolar spaces was greater and interstitial edema became evident. These data suggest that a transmural pressure threshold necessary for pulmonary capillary stress failure exists between 75 and 100 mmHg, and thus any reduction in Ppa in this range has the strong likelihood of substantially reducing pulmonary capillary stress failure-induced EIPH as evidenced in the present investigation.

Thus, although the results of the present investigation appear to be consistent with the previous work described above, why has previous research not shown definitively that Fur is effective in reducing EIPH? The answer appears to be due, in part, to the techniques for assessment of EIPH and the manner in which they have been used to assess the efficacy of Fur.

**Techniques for assessment of EIPH.** Present methods for assessment of EIPH include tracheal endoscopy, scintigraphy, and BAL, all of which have been viewed with a healthy degree of skepticism (3, 11). In the present investigation, BAL techniques were used to assess the severity of EIPH. This technique has been criticized on the basis of accuracy and reproducibility (11). However, our laboratory has found that, under controlled, laboratory conditions in which horses run identical protocols, the recovery of BAL fluid and the number of RBC per milliliter of BAL fluid is highly reproducible between runs (i.e., coefficient of variation = ~5%). Furthermore, in one horse that performed three paired incremental trials with and without the NS, the reduction in EIPH assessed via BAL was consistent (i.e., ~77 and ~79% to 15 m/s and ~40% when following protocol to 14 m/s treadmill speed), thus suggesting that BAL can be an effective tool to study the effects of experimental interventions on the EIPH response under a controlled, treadmill laboratory setting. Indeed, the mean reduction in EIPH with the NS between this investigation (40%) and that of Poole et al. (33%; Ref. 25) are closely comparable. Although it is certainly true that one limitation of the BAL technique is that global lung bleeding cannot be assessed, placement of the Bivona tube in the subsegmental bronchi (19) of the dorsal caudal region of the lung (i.e., the place where the majority of EIPH is incurred; Refs. 17, 21) offers the next best alternative to removal of the entire lung for whole lung lavage. Other techniques used to assess EIPH are endoscopy and scintigraphy. Scintigraphic imaging has proved ineffective because of the inability to differentiate between extravascular and intravascular cells (31). Tracheal endoscopic evaluation, the most common technique used to assess EIPH, also has many inherent limitations. The anatomical relationship between the site of bleeding and the site of observation, uncertainty regarding the efficiency of mucociliary clearance, and the subjective nature of scoring blood in the airways on a scale of 1 to 4 all limit the capacity to assess EIPH severity quantitatively and objectively. Indeed, from the work of Meyer et al. (20), the reductions in EIPH reported in the present investigation would likely have gone undetected if assessed via tracheal endoscopy.

**Previous findings.** Studies in which the incidence of EIPH has been assessed (via endoscopy) after Fur use on the track have, in general, shown minimal/no reduction in EIPH incidence and whether Fur use reduces EIPH severity has remained undetermined (23, 28, 30). Certainly, studies on the track conducted to assess whether Fur reduces the severity of EIPH are equivocal, some having shown a reduced endoscopy score (24) and others not (29). Again, these disparate findings can likely be attributed to the shortcomings in the sensitivity of the endoscopy technique as well as day-to-day variations on the track that cannot be controlled. As discussed in the INTRODUCTION, to our knowledge the only study using BAL techniques to assess EIPH was that of Lester et al. (14). Possible limitations in that investigation were discussed above. However, it is worth noting that the horse in our investigation with the lowest EIPH (far left, Fig. 2B) had a value close to the mean reported by Lester et al. and did not demonstrate any reductions with NS and/or Fur. It is appar-
ent that individual horses not suffering from severe EIPH after strenuous running would not be expected to receive much benefit from any intervention.

In conclusion, both NS and Fur reduced EIPH severity as assessed via RBC counts in BAL fluid; however, Fur was more effective. Neither NS nor Fur abolished EIPH after strenuous running would not be expected to receive much benefit from any intervention. The Fur-induced decrease in pulmonary vascular pressure provides one likely mechanism for reducing EIPH severity. Surprisingly, NS did not alter the magnitude of the pressure swings, and hence its mechanism of action remains to be determined. In the present investigation it was not feasible to determine lung volumes or measure intrapleural pressure directly, and in this regard further studies are warranted.

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