Effect of Dexamethasone and Fluticasone on Airway Hyperresponsiveness in Horses With Inflammatory Airway Disease

R. Léguillette (iD), T. Tohver, S.L. Bond (iD), J.A. Nicol, and K.J. McDonald

Background: Airway hyperresponsiveness (AWHR), expressed as hypersensitivity ($PC_{75}R_L$) or hyperreactivity (slope of the histamine dose-response curve), is a feature of inflammatory airway disease (IAD) or mild equine asthma in horses. Glucocorticoids are used empirically to treat IAD.

Objectives: To determine whether dexamethasone (DEX) (0.05 mg/kg IM q24h) and inhaled fluticasone (FLUT) (3,000 µg q12h) administered by inhalation are effective in decreasing AWHR, lung inflammation, and clinical signs in horses with IAD.

Methods: A randomized crossover study design was used. Eight horses with IAD were assigned to a treatment group with either DEX or FLUT. Measured outcomes included lung mechanics during bronchoprovocative challenges, bronchoalveolar lavage fluid (BALF) cytology, and scoring of clinical signs during exercise.

Results: Dexamethasone and FLUT abolished the increase in R_L by 75% at any histamine bronchoprovocative dose in all horses after the first week of treatment. However, after 2 weeks of FLUT treatment, 1 horse redeveloped hypersensitivity. There was a significant decrease in the number of lymphocytes after treatment with both DEX and FLUT ($P = .039$ for both) but no significant differences in other BALF cell types or total cell counts ($P > .05$). There was no difference in the scoring of the clinical signs during each treatment and washout period ($P > .05$).

Conclusions and Clinical Importance: Both DEX and FLUT treatments significantly inhibit airway hypersensitivity and hyperreactivity in horses with IAD. There are no significant effects on the clinical signs or the number of inflammatory cells (except lymphocytes) in BALF. The treatments have no residual effect 3 weeks after discontinuation.

Key words: Airway hyperreactivity; Airway hypersensitivity; Bronchoalveolar lavage; Equine asthma; Histamine bronchoprovocation challenge; Respiratory clinical signs.

M ild equine asthma (inflammatory airway disease [IAD]),[1] together with recurrent airway obstruction (RAO) and summer pasture-associated obstructive pulmonary disease, comprise the equine inflammatory respiratory diseases.[1–3] The prevalence of IAD is high and can affect horses of any age and discipline.[4] It can impact performance in both racehorses and sport horses.[1] Inflammatory airway disease is defined as a noninfectious inflammatory lung disease with 3 predominant traits: (1) respiratory clinical signs at work, exercise intolerance without clinical signs of labored breathing at rest, or both, even after exposure to moldy hay; (2) evidence of pulmonary dysfunction in the form of airway hyperresponsiveness (AWHR); (3) nonseptic inflammation based on bronchoalveolar lavage (BAL) cytologic evaluation.[1] Airway hyperresponsiveness is one of the main features of IAD and can contribute to the development of clinical signs. Analogous to human asthma, AWHR can be objectively and reliably demonstrated in horses using bronchoprovocative challenge

Abbreviations:

AWHR	airway hyperresponsiveness
BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
DEX	dexamethasone
E_L	pulmonary elastance
FLUT	inhaled fluticasone
IAD	inflammatory airway disease (mild equine asthma)
$PC_{75}R_L$	airway hypersensitivity
RAO	recurrent airway obstruction (severe equine asthma)
R_L	pulmonary resistance

with histamine.[5–7] This allows measurement of airway sensitivity (threshold of the bronchoconstriction response) and reactivity (magnitude of the bronchoconstriction response). Airway hyperresponsiveness is a valuable variable for both research and clinical practice because it can be detected before more obvious clinical signs develop.[8] An increased number of mast cells in the BAL fluid,[8–10] respiratory clinical signs, and exercise intolerance[7,11] are correlated with AWHR in horses.

The exact etiology of IAD is still unknown. Several studies have demonstrated a link between IAD and a poor environment.[1,8,12–14] There is also evidence that supports allergy as a contributing factor for the disease.[8,15] A connection between infectious airway disease with tracheal inflammation in young racehorses[16,17] and IAD has been suggested. Although many studies have been published on the diagnosis and characterization of the phenotype of IAD in horses, the scientific evidence for treatments of IAD is extremely limited. More recently, 1 study showed that dietary supplementation with Omega-3 with environmental modifications and lung

inflammation will be controlled more rapidly than with only environmental modification.[18] Despite this gap in our knowledge and because IAD is an inflammatory lung disease, it is common practice to treat IAD with glucocorticoids. In several studies on heaves (RAO), dexamethasone (DEX) has specifically been used as a reference treatment to which other glucocorticoids have been compared.[19–21] Both DEX and inhaled fluticasone (FLUT) are effective in relieving clinical signs and significantly decreasing neutrophilia in bronchoalveolar lavage fluid (BALF) in horses with severe asthma (RAO).[20–22]

The objective of this study was thus to evaluate and compare the effects of DEX and FLUT on the clinical signs, AWHR and BAL fluid cytology in horses with IAD. We hypothesized that both glucocorticoids would improve clinical signs and lung function as well as alter the cytologic findings of BALF.

Material and Methods

This study was approved by the Animal Care Committee of the Health Science Centre at the University of Calgary. The authors used the REFLECT statement guidelines to report this study.[23]

Horses

Eight adult horses (median body weight 512 kg; range 434–563 kg) with IAD from our research herd were studied. The number of horses was calculated using a power of 0.9 for a difference in measured variables between baseline and treatments of 2 times the within-patient standard deviation.[a] Horses were various breeds, predominantly Quarter Horses or Thoroughbreds, 4 mares, and 4 geldings of various ages (4–16 year old). Criteria for inclusion were as follows: (1) the presence of respiratory clinical signs during exercise without labored breathing at rest, (2) the absence of increased lung resistance at rest after a challenge with moldy hay,[24] (3) the presence of AWHR measured by an increase in lung resistance (R_L) by 75% at lower doses of nebulized histamine,[25] (4) a BAL with increased percentage of mast cells (>2%) or/and eosinophils (>0.1%) or/and neutrophils (>5%).

Prior to the experiment, horses were conditioned to stand in stocks wearing a mask. The animals were kept in the same outside paddocks for at least 3 weeks before the experiment and the management remained the same throughout the period of the study. The horses were kept on straw and were fed round bale hay and a pellet supplement. None of the horses received treatment for respiratory disease during the 3 months preceding the study.

Bronchoalveolar Lavage

Bronchoalveolar lavages were performed in the morning (8:00–10:00 AM) using a standard protocol.[4] Briefly, horses were sedated with xylazine[b] (0.8–1.0 mg/kg of body weight, IV) and butorphanol[c] (10–20 μg/kg of body weight, IV). A videoendoscope (3 m length, 12.9 mm diameter) was then inserted through the nostrils and directed down into the left lung until its tip was wedged in a distal bronchus. Small boluses of 0.5% lidocaine[d] solution were administered (up to a maximal volume of 120 mL) to desensitize the airway mucosa. Two 250-mL boluses of sterile 0.9% sodium chloride were alternatively instilled under pressure into the bronchus and aspirated via the endoscope biopsy channel by use of a suction pump. The BAL fluid was collected in a 500-mL plastic Nalgene jar and its volume was recorded. A 5-mL sample of the BAL fluid was immediately put into a Vacutainer

EDTA tube which was stored on ice until analysis. Cytology slides were prepared within 3 hours of BAL procedure using a cytospin (113 g for 4 minute), then stained with an automatic stainer[e] using a Modified Wright Giemsa solution for better visualization of mast cells. Differential counts were performed on at least 400 nucleated cells, not including epithelial cells, by 1 author (NF, clinical pathologist), who was blinded to all the results of the study.[26]

Lung Function Tests

Baseline lung mechanics measurements and histamine challenges were performed on the horses as previously described, with modifications.[6,27–29] Briefly, standard lung mechanics were measured in unsedated horses before and during the bronchoprovocative challenge using airflow and esophageal pressure data acquisition. Flow rate was measured by a heated pneumotachograph[f] attached to a custom-made fiberglass mask sealed over the nose of each horse. Transpulmonary pressure (P_L) was obtained by use of a differential pressure transducer,[g] which was connected to a small-diameter esophageal tube (inside diameter, 2 mm; outside diameter, 4.5 mm) with a balloon sealed over the end and placed in the distal third of the esophagus. The second port of the differential pressure transducer was connected to the mask to subtract the mask pressure from the esophageal pressure. The balloon was distended with 15 mL of air and positioned to obtain the maximal changes in P_L during a respiratory cycle (ΔP_L) and to eliminate cardiac artifacts. The balloon was checked for leaks at the beginning and at the end of each experiment. The system was calibrated for each experiment using a calibrated 3-L syringe[h] and a water manometer for the flow and pressure signals, respectively. The signals from the transducers were processed and analyzed using the UnitWise and Flexiware data acquisition and analysis system.[i] In addition to spirometry variables, values of pulmonary resistance (R_L) and elastance (E_L) were calculated at a rate of 200 Hz by applying the data to the multiple regression equation for the single-compartment model of the lung.[27] The coefficients of determination for the fit of the equation to the data were calculated.

Airway hyperresponsiveness was evaluated using histamine bronchoprovocation. Briefly, once baseline measurements were calculated based on an average obtained over a minimum of 20 consecutive breaths at steady state, lung mechanics were assessed after nebulization with saline and increasing doses of histamine[j] (1, 2, 4, 8, 16, and maximum 32 mg/mL). Each dose was administered for 90 seconds through a fine-particle jet nebulizer[k] (0.5 mL/min) powered by a high-pressure (30 psig), high-flow (9 L/min) air compressor.[l] A connector system with an aerochamber[m] and 1-way valves was attached between the nebulizer and the facemask. After each nebulization, the connector system was immediately removed from the mask and replaced with the pneumotachograph for data collection. The test was terminated either when the pulmonary resistance (R_L) doubled compared to the baseline resistance or when the maximum histamine dose (of 32 mg/mL) was delivered.

Concentration-response curves were plotted for each bronchoprovocative challenge test as the percentage increase in R_L from baseline against the histamine concentration (Fig 1). Airway hypersensitivity and reactivity (slope of the concentration-response curve) were determined as follows (Fig 1): The dose of histamine that evoked a 75% increase of baseline R_L ($PC_{75}R_L$), which is an indicator of airway sensitivity,[25] was determined by interpolation or extrapolation of the histamine dose-response curve depending on the increase in R_L compared to baseline. In horses for which R_L crossed the 75% increase threshold before the maximal dose (32 mg/mL) of nebulized histamine, the $PC_{75}R_L$ was determined by interpolation of the line between the last 2 points of the concentration-response curve (A–B in Fig 1). In horses for which R_L

stayed lower than the 75% increase threshold value for all doses of nebulized histamine, a linear regression of the last points (2 or 3, depending on which resulted in a positive value or a more even plateau) of the curve was used and the $PC_{75}R_L$ value was determined by extrapolation of the line. In addition, if the slope of the last points of the line was negative, we conservatively set the $PC_{75}R_L$ at 32 mg/mL. Lastly, we calculated airway reactivity by calculating the slope of the concentration-response curves using the same points as for $PC_{75}R_L$. Because the baseline value is an important point for the calculations, we averaged the R_L values of baseline and saline and also used a linear regression of the first 3 points of the histamine concentration-response curve (averaged pre-post saline, 1 and 2 mg) to determine baseline R_L values (Fig 1).

Clinical Signs

We modified a previously described clinical score[10] to grade respiratory clinical signs before, during, and after exercise (Table 1). The horses were lunged in an arena with side reins after an exercise protocol of 1-minute walk, 7-minute trot, and 1-minute canter. The arena had a sand and rubber chip footing that was watered for 15–20 minutes prior lunging to minimize dust exposure. The lunging and evaluation of clinical signs were performed by 1 author (TT) who was not blinded to the study results. All horses tolerated the exercise well and could be lunged according to plan throughout the study. Before exercise, the horse's rectal temperature was recorded to exclude infectious respiratory disease. We first scored breathing effort, nostril flare, and nasal discharge before and during lunging. Then, immediately after the canter period, the respiratory rate was recorded for 1 minute and a photograph of both nostrils was taken. Nasal discharge was scored based on the area of the nostril (average of both nostrils) covered with mucus and on its distribution on the upper lip (Table 1B). The number of coughs was also counted throughout the exercise. This scoring system has yet to be validated for IAD; however, the presence of a chronic cough (>3 weeks duration) and nasal discharge can indicate an increased risk for developing IAD[1]; the scoring system used is described in Table 1.
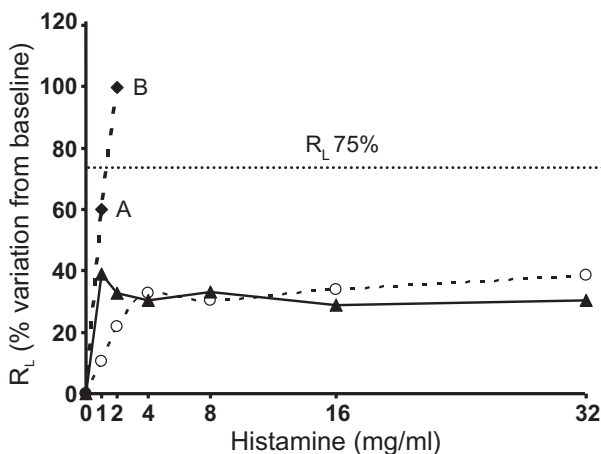
Experimental Protocol

The study used a controlled randomized crossover design. Randomization was performed by one author (RL) using Microsoft Excel Random Generator function. Two groups with 5 and 3 horses each were subjected to 2 treatment protocols separated in the middle by a washout period. On day −1 of the study, a BAL was performed on all the horses as described above. On day 0, approximately 24 hours after the BAL, baseline lung mechanics and histamine bronchoprovocation challenge were carried out as described above. The treatments with DEX and FLUT were started on day 1 of the study. Dexamethasone[n] (0.05 mg/kg) was administered intra muscularly once a day in the morning (7:00–8:00 AM) for 15 days. Fluticasone propionate[o] (3,000 μg) was administered using metered dose inhalers (MDIs) and an Aerohippus[p] twice daily (7:00–8:00 AM and PM) for 15 days. Lung mechanics and histamine bronchoprovocation challenges were performed on days 8 and 16. A second BAL was performed on day 15. The first treatment phase was followed by a 3-week washout period before switching to the second treatment, for which the same protocol was followed. The horses were lunged, starting on day 1 of the study, every second day during the treatment and every fourth day during the washout period. Day 36, which was the last day of the second washout period, was also the last day of lunging for both groups (Table 3).

Statistical Analysis

Nonparametric tests (Wilcoxon signed-rank test) were used to compare airway hyperreactivity, hypersensitivity, and BAL variables between treatments as well as before and after each treatment. A Friedman 2-way test was used to assess variation in clinical scores over each treatment and washout period, with Bonferroni correction for multiple testing of clinical scores used to determine level of significance for P values. A Spearman rank correlation was used to test for correlation between pulmonary sensitivity or reactivity and BAL cytological parameters. Values were expressed as the median (1st Quartile–3rd Quartile). A P value < .05 was considered significant (lower when Bonferroni correction was applied). Statistical analysis was carried out using commercial software.[q]

Results

Lung mechanics, histamine bronchoprovocation challenges, BAL, and lunging procedures were well tolerated by all horses. Seven horses completed the study. One of the 8 horses could only be used in the DEX treatment phase because she was euthanized due to femoral nerve paresis during the FLUT treatment phase (causality unrelated to study).

Histamine Bronchoprovocation Challenges: Airway Hypersensitivity and Hyperreactivity

The coefficients of determination for the regression analysis used to calculate R_L and E_L during the lung mechanics experiments for DEX and FLUT, respectively, had a median value of 0.95 (0.92–0.97) and 0.95 (0.92–0.97). All horses had airway hypersensitivity at baseline prior to DEX and FLUT treatments, as shown by the low baseline $PC_{75}R_L$ values (Fig 2). The median $PC_{75}R_L$ values were 6.7 mg/mL (5.1–13.4) and 14.2 mg/mL (7.6–24.7) at baseline before DEX and FLUT

Fig 1. Example of concentration-response curves obtained in 1 horse before (♦), after 8 days (o), and 15 days (▲) of treatment during the histamine bronchoprovocation challenge. Percentage increase in R_L is compared to baseline R_L. The dotted line shows the threshold for increased R_L values by 75%. A and B show the 2 points used to calculate the $PC_{75}R_L$ by interpolation.

Table 1. Clinical scoring system for respiratory signs in horses with inflammatory airway disease: (A) Clinical scoring system for respiratory signs during and after exercise; (B) Details on the scoring of nasal discharge.

(A)

Respiratory effort[a]	**0**- normal	**1**- mildly increased	**2**- moderately increased	**3**- severely increased
Respiratory rate[a]	**0**- ≤48	**1**- ≥48≤64	**2**- ≥64	
Nostril flare[a]	**0**- none	**1**- moderate	**2**- severe	
Nasal discharge[a,b]	**0**	**1**	**2**	**3**
Nasal discharge increase[c]	**0**- no increase	**1**- increase by 1	**2**- increased by ≥2	
Cough[d]	**0**- none	**1**- 1 or 2 coughs	**2**- 3 coughs	**3**- 4 or more coughs
Total score: 0–15				

Score	Nostril[e]		Upper lip
(B)			
1	<⅓	±	1–2 thin streams
2	⅓–⅔	±	≤2 finger wide stream
3	>⅔	±	>2 finger wide stream

[a]Scored after exercise (1 minute duration).
[b]See Table 1B for details on the scoring of nasal discharge.
[c]Difference between before and after exercise.
[d]Cough counts during exercise.
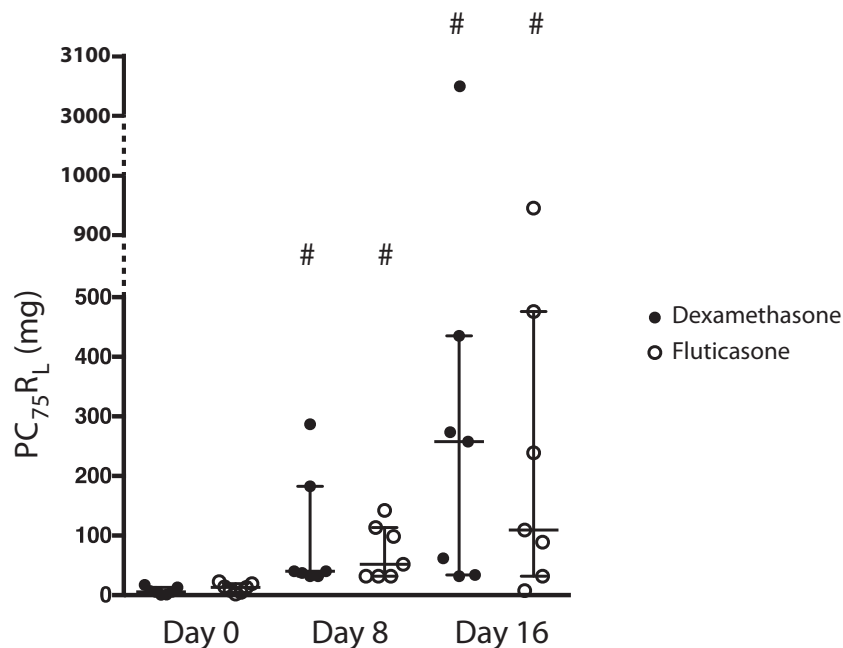[e]Area of the nostril covered with mucus. The mean value of both nostrils is used.

Fig 2. Whisker plot with individual values of $PC_{75}R_L$ before treatment (Day 0), the day after 1 week (Day 8), and the day after 15 days (Day 16) of treatment with intramuscular dexamethasone (filled circles) and inhaled propionate fluticasone using metered dose inhalers (open circles). # indicates significantly different from respective baseline value. Large bars indicate median values, and smaller bar at the bottom and top indicate 1st and 3rd interquartile values.

treatments, respectively (Fig 2). There was no significant difference ($P = .23$) in the values of R_L between treatment baselines for DEX and FLUT (Fig 2).

The DEX treatment abolished the 75% increase in R_L from baseline at any dose of histamine used in the bronchoprovocation challenge 8 and 16 days after initiation of treatment in all 8 horses. The calculated $PC_{75}R_L$ median values were 40.2 mg/mL (32.0–182.8) and 257.7 mg/mL (33.8–435.2) after 8 and 16 days of

DEX treatment, respectively, which were both significantly different from treatment baseline ($P = .01$ for both) (Fig 2). There was no significant difference in $PC_{75}R_L$ between 8 and 16 days of treatment with DEX ($P = .15$) (Fig 2).

The FLUT treatment abolished the 75% increase in R_L from baseline at any dose of histamine used in the bronchoprovocation challenge after 8 days of treatment in all 7 horses but in 6 horses after 16 days of
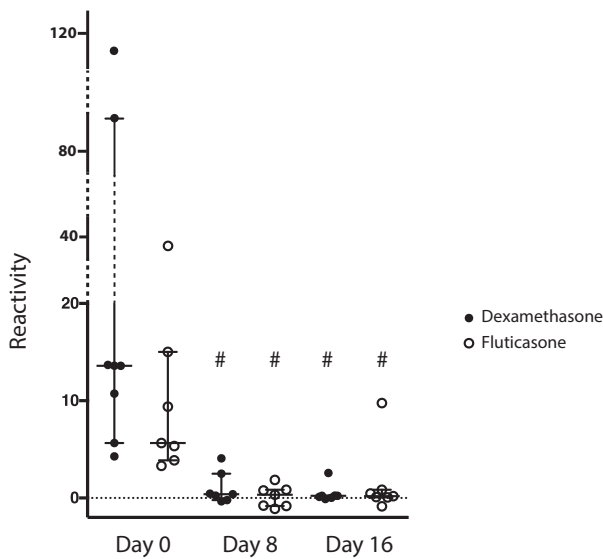
Fig 3. Whisker plot with individual values of airway reactivity before treatment (Day 0), the day after 1 week (Day 8), and the day after 15 days (Day 16) of treatment with intramuscular dexamethasone (filled circles) and inhaled propionate fluticasone using metered dose inhalers (open circles). # indicates significantly different from respective baseline value. Large bars indicate median values, and smaller bar at the bottom and top indicate 1st and 3rd interquartile values.

Fig 4. Comparison of the effects of intramuscular dexamethasone and inhaled propionate fluticasone on airway reactivity in 7 horses. Values are shown before and after 15 days or treatment with dexamethasone (**A**) and fluticasone (**B**).

treatment. One horse redeveloped hypersensitivity ($PC_{75}R_L$ = 2.5 mg/mL) after 16 days of FLUT treatment. The calculated median $PC_{75}R_L$ values were 52.0 mg/mL (32.0–113.4) and 109.2 mg/mL (32.0–475.9) after 8 and 16 days of FLUT treatment, respectively, which were both significantly different from baseline (P = .01, and P = .02, respectively) (Fig 2). The difference in $PC_{75}R_L$ between 8 and 16 days of treatment with FLUT was not significant (P = .078) (Fig 2).

All horses showed increased concentration-response curve slope values at day 0 in both DEX and FLUT treatments (13.6 (5.7–82.9) and 5.3 (3.3–9.4), respectively), indicative of airway hyperreactivity (Fig 3). There was no significant difference (P = .15) in the slope values between baselines for DEX and FLUT (Fig 3). Compared to baseline, the slope values of the concentration-response curve decreased significantly at day 8 and day 16 for both DEX (P = .008 for both) and FLUT treatments (P = .008 for both) (Figs 3 and 4). There was no significant difference in the slope values between 8 and 16 days of treatment with DEX and FLUT (P = .50 and P = .41, respectively).

Bronchoalveolar Lavage Cytology

The BALF of all the horses showed neutrophilic inflammation before each treatment (Table 2). Additionally, 4 horses before DEX treatment and 5 horses before FLUT treatment, respectively, had an increased number of mast cells. There was no association between the types of inflammation (neutrophils, mast cells, eosinophils' percentage) in BALF of individual horses and the results of the bronchoprovocative tests ($PC_{75}R_L$ and
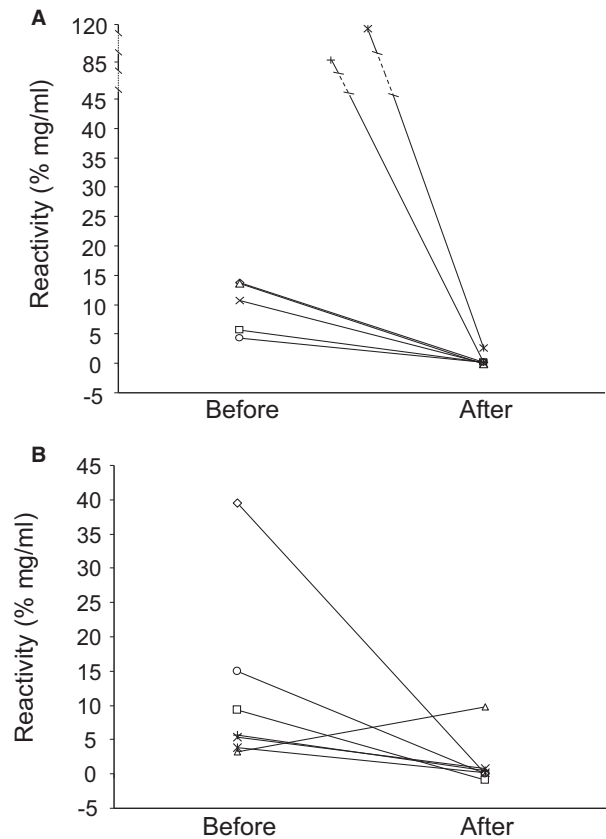
reactivity). There was no significant difference between the DEX and FLUT treatment baseline values for the BAL fluid total cell counts or differential cell counts (Table 2). The lymphocyte percentage decreased significantly in the BAL fluid after both DEX and FLUT treatments (P = .039 for both) (Table 2). There was no significant difference in total cell count or differential cell count for any other cell type in the BAL fluid between before and after treatment with DEX and FLUT (Table 2) although there was an evident trend in the decrease of mast cells after both treatments. The BAL sample volume collected after treatment with FLUT was significantly greater than the baseline BALF volume (P = .031); however, there was no significant increase in BALF volume after treatment with DEX (Table 2).

Clinical Signs Score

There was no significant difference in clinical scores at baselines between the DEX and FLUT treatments. There was no significant change in the total clinical score of the horses over time (Table 3A,B). When analyzing each clinical variable separately, namely, respiratory effort, nasal discharge, increase in nasal discharge with exercise, nasal flare, coughing, and respiratory rate

Table 2. Median (1st Quartile–3rd Quartile) values for cytologic evaluation of bronchoalveolar fluid obtained before (Day 0) and after 15 days of treatment with intramuscular dexamethasone (DEX) (0.05 mg/kg q24h) (8 horses) and inhaled propionate fluticasone (3,000 μg q12h using metered dose inhalers) (7 horses), respectively.

Variable	DEX		Fluticasone	
	Day 1	Day 15	Day 1	Day 15
Total nucleated cells (No./μL)	150 (93–299)	162 (57 –212)	122 (97–247)	123 (36–143)
Neutrophils (%)	31.9 (23.7–40.2)	20.5 (12.7–64.0)	12.5 (6.8–24.5)	23.5 (7.8–54)
Mast cells (%)	2.5 (1.3–2.8)	1.25 (0.7–2.0)	2.5 (1.5–3.3)	1.3 (1.0–1.5)
Eosinophils (%)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Lymphocytes (%)	41.5 (36.3–49.8)	33.7 (17.0–45.5)[a]	53.7 (47.7–63.7)	40.3 (27.3–59.5)[a]
Macrophages (%)	25.4 (13.5–26.5)	38.7 (18.5–49.8)	25.2 (14.5–40.3)	29.3 (13.3–33.0)
Volume (mL)	300 (250–310)	280 (250–325)	250 (190–260)	300 (250–400)[a]

[a]Significantly different from Day 1 ($P < .05$).

Table 3. Median (1st Quartile–3rd Quartile) values of clinical scores obtained from (a) 8 horses during days of treatment with dexamethasone (DEX) and the subsequent washout period and (b) 7 horses (see Methods) during days of treatment with fluticasone propionate using metered dose inhalers and the subsequent washout period.

(A)	Treatment Days (DEX)						
	1	3	5	7	9	11	13
Cough	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–0.25)	0 (0–0)	0 (0–0)	0 (0–0.25)
Respiratory effort	1.5 (1–2)	2 (1–2)	1.5 (1–2)	2 (2–2)	2 (1–2)	2 (1–2)	1 (1–2)
Respiratory rate	0 (0–0)	0 (0–2)	0 (0–2)	0 (0–0)	0 (0–1)	0 (0–0)	0 (0–0)
Nostril flare	1 (0.75–1.25)	1 (0.75–1.25)	1 (0–2)	1 (0.75–1.25)	1 (0.75–1.25)	1 (1–1)	1 (0–1)
Nasal discharge	2 (1 –2)	1.5 (1–2)	1.5 (1–2)	1.5 (1–2)	1 (1–2)	1 (1–1)	1.5 (1–2)
Nasal discharge increase	1 (0–1)	0 (0–1)	0.5 (0–1)	0.5 (0–1)	0 (0–1)	0 (0–0)	0.5 (0–1)
Total score	4.5 (3.75–6.25)	6 (3.5–7.25)	6 (4.5–7.5)	5 (3.75–6.75)	4.5 (3.75–6.25)	3.5 (3–4)	3.5 (3–5.5)

	Washout Days						
	16	18	22	26	30	34	36
Cough	0 (0–0.25)	0 (0–1)	0 (0–1.5)	0 (0–0)	0 (0–0.25)	0 (0–0.25)	0 (0–0.25)
Respiratory effort	2 (1–2)	2 (1.75–2)	1 (1–1.25)	1 (0.75–1.25)	1.5 (1–2)	1.5 (0.75–2)	2 (1.5–2.5)
Respiratory rate	0 (0–0.25)	0 (0–0.25)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–1)
Nostril flare	1 (1–1)	1 (0–1)	0.5 (0–1)	0 (0–1.25)	0.5 (0–1)	0 (0–1)	1 (0–1)
Nasal discharge	1.5 (1–2)	2 (1.75–2)	1.5 (1–2)	2 (2–2)	1 (1–1.25)	1.5 (1–2)	1 (1–1.25)
Nasal discharge increase	0 (0–1)	1 (0.75–1)	1 (0–1)	1 (1–1)	0 (0–0.25)	1 (0–1)	0 (0–1)
Total score	5 (3–6.5)	6 (4.5–7)	5 (3.75–5.25)	4.5 (4–5.25)	4 (2.75–4.25)	4 (2–5.5)	4.5 (2–6.25)

(B)	Treatment Days (Fluticasone)						
	1	3	5	7	9	11	13
Cough	0 (0–0.5)	0 (0–0)	0 (0–0.5)	0 (0–0)	0 (0–0.5)	0 (0–0)	0 (0–0)
Respiratory effort	2 (2–2)	2 (1–2)	2 (1–2)	2 (1–2)	1 (1–2)	1 (1–2)	2 (1–2)
Respiratory rate	0 (0–1)	0 (0–0)	0 (0–1)	0 (0–0.5)	0 (0–0)	0 (0–0)	0 (0–0.5)
Nostril flare	1 (0–1.5)	1 (0.5–1)	1 (0.5–1)	1 (1–1)	1 (1–1)	1 (0.5–1)	1 (1–1)
Nasal discharge	1 (1–2)	2 (2–2)	1 (1–2)	1 (1–1.5)	1 (1–2)	2 (1–2)	1 (1–2)
Nasal discharge increase	0 (0–1)	1 (1–1)	0 (0–1)	0 (0–0)	0 (0–0.5)	1 (0–1)	0 (0–0.5)
Total score	5 (4–7.5)	5 (5–6.5)	5 (4.5–5.5)	4 (3–5.5)	4 (3–6)	4 (3–5.5)	5 (3.5–5.5)

	Washout Days						
	16	18	22	26	30	34	36
Cough	0 (0–1)	1 (0–1.5)	1 (0–1)	1 (0–1.5)	0 (0–1)	0 (0–0.5)	0 (0–1)
Respiratory effort	1 (1–2)	2 (1–2)	2 (1–2)	1 (1–1.5)	1 (0–2)	2 (1–2)	1 (0.5–1)
Respiratory rate	0 (0–0)	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Nostril flare	1 (0.5–1)	1 (0–1)	1 (0–1)	1 (0–1)	1 (1–1)	1 (1–1)	1 (0.5–1.5)
Nasal discharge	1 (1–1)	1 (1–2)	1 (1–1.5)	1 (1–2.5)	2 (1.5–2.5)	2 (1–2)	1 (1–1.5)
Nasal discharge increase	0 (0–0)	0 (0–0.5)	0 (0–0.5)	0 (0–1.5)	1 (0.5–1)	0 (0–1)	1 (0–1)
Total score	3 (2.5–5)	5 (4–6)	4 (2.5–5.5)	5 (3–7)	5 (4–6)	4 (4–5.5)	4 (3–5.5)

(see Tables 1 and 3A,B), there was no statistically significant change across all times points for both treatments (increase in nasal discharge for DEX and FLUT treatment had P values of .046 and .029, respectively, respiratory rate and nasal discharge for FLUT treatment had P values of .023 and .042, respectively, which were all nonsignificant after Bonferroni correction for multiple testing of clinical scores).

Discussion

The main finding of the present study is that both DEX and FLUT treatments significantly decrease airway hypersensitivity and hyperreactivity in horses with IAD (mild equine asthma). Dexamethasone was administered intramuscularly in this study because it is typically used in practice to ensure adequate dosage while avoiding the need for a catheter or trained personnel for the administration of the medication. No side effects were observed. Aerosol steroid therapy is commonly used in equine medicine; we administered FLUT using MDIs and the Aerohippus device, which is more practical than the Aeromask and has been shown to have a better diffusion of drug particles in the lower airways.[30] Horses tolerated the aerosol therapy very well.

Airway hyperresponsiveness is a well-described feature of human asthma[31,32] and has also been described numerous times in horses with nonseptic respiratory diseases.[1,5,7,8] In healthy human subjects, airway reactivity reaches a plateau or maximal response, where the airway smooth muscles are activated maximally. In subjects with asthma, the plateau response is increased or abolished altogether, as there are no more limitations to maximal airway narrowing.[33] These variables are believed to have independent etiologies although the correlation between the 2 remains unclear.[34] To our knowledge, this distinction between airway reactivity and sensitivity has not previously been characterized in equine airway diseases. There have been minimal references to the plateau response in veterinary studies[8,35] and it is uncertain whether a plateau as seen in human patients also exists in horses. The fact that an abolished plateau response in humans can lead to fatal asthma incidents and that airway hyperreactivity in horses typically does not result in death suggests that the mechanisms behind airway reactivity are different or more severe in humans than in horses.

In this study, we looked at reactivity and sensitivity as separate features of airway responsiveness as well as at a possible plateau response in horses. There is no reference to the physiological slope values (ie, airway reactivity) of the concentration-response curve in horses. Therefore, we evaluated the changes in the values of this variable during treatment periods but did not use the airway reactivity as an inclusion criterion for horses into the study. However, based on the results of this study, we would suggest that a reactivity value of more than 5 (% mg/mL) is indicative of airway hyperreactivity and a value <2 (% mg/mL) can be regarded as normal. Further studies are necessary to validate and establish normal values for airway reactivity in horses.

In a previous study on horses, a plateau was defined as a change in R_L of <10% after 3 consecutive doses of histamine.[8] We could observe a plateau in the majority of the horses with airway hypersensitivity inhibited by the therapies but also witnessed a repeated increase in R_L with higher doses of histamine after a plateau had been reached in 5 horses.[8] This observation of R_L fluctuations of more than 10% during histamine bronchoprovocation could mean that higher doses of histamine are needed to provoke a stable plateau response in horses or that horses do not have a maximal plateau response to specific agonists comparable to humans. We did not use the plateau response in the statistical analysis of our study due to the yet uncertain value of this variable in horses.

Although DEX and MDIs FLUT treatments decreased airway hypersensitivity and reactivity in these horses with IAD, they did not affect the total or the differential cell counts of the BAL fluid. The persistent lower airway inflammation measured by the BAL technique in our study was similar to previous studies on the treatment of RAO in horses with steroids.[27,36,37] Conversely, other studies have shown a decrease in the amount of inflammatory cells after steroid treatment.[21,22] The environmental conditions were not changed in our study, which might contribute to the persistent accumulation of inflammatory cells in the lower airways. However, the lack of a negative control group treated with a placebo in the study does not allow conclusions to be made on the effect of the environment on lung hypersensitivity and inflammation in these horses. We did not find any significant association between types of inflammation in the BALF (neutrophilic, mast cell, eosinophilic) and AWHR in individual horses as has been reported previously.[9] However, this might be due to a lack of power and from the crossover study design that included a small number of horses with each type of inflammation (and none with eosinophilic inflammation). There was a noticeable trend in the decrease of mast cells after both treatments which might have been significant had the environmental conditions been changed in the study. Bronchoalveolar lavage cytology is a good method for measuring the number of inflammatory cells in the lung but it does not give information about the activation level of the various cell types found in the lower airways. Therefore a decrease in airway hypersensitivity and reactivity in spite of a persistent high percentage of inflammatory cells in the lower airways could be due to a decreased activation level of the inflammatory cells. It is also possible that, similarly to human asthma, steroids inhibit neutrophil apoptosis in horses with IAD,[38] thus maintaining greater levels of inflammatory cells in the airways.

Inflammatory airway disease is also defined by poor performance, exercise intolerance, or coughing, with excessive tracheal mucus.[1] These variables have been previously evaluated in horses either by measuring gas exchange[39,40] and metabolic response to exercise during a treadmill test or by subjective evaluation of the horses performance and competition results.[8] The challenge in evaluating clinical signs in nonracehorses is the lack of

reference values and standardized tests for horses with different fitness levels and aerobic capacities. In this study, we modified a previously described scoring system[10]; we evaluated each clinical variable separately, and then added them to calculate the comprehensive clinical score during treatment and washout periods (Tables 1 and 3). The increase in nasal discharge induced by exercise was the only variable that showed a significant increase in the washout period after DEX treatment (Table 3). This result suggests that airway inflammation increased during the washout period after the DEX treatment. Other clinical variables remained largely unchanged throughout the study. This might be due to a lack of sensitivity from our scoring system, possibly due to an exercise intensity that was not great enough to reveal the clinical differences induced by the treatments. Another difficulty in objectively measuring clinical variables is the influence by environmental factors like weather, dust, or chemical irritants as well as potentially by coincidental factors like head position, previous coughing, or time of the day. Further research is needed to validate objective clinical scoring for submaximal exercise conditions in horses with IAD.

Although there is evidence that airway inflammation is associated with AWHR,[6,8,10,41] the correlation between AWHR and respiratory clinical signs has not been established in horses. The importance of the association between these 2 traits in IAD is largely unknown. The fact that both glucocorticoids in our study significantly decreased AWHR but did not alter the BAL cytology (excepting lymphocyte count) nor change clinical signs might mean that these features of IAD have different etiologies or pathophysiology and also have to be addressed separately in treatment. In our opinion, these results reflect the complex nature of the disease and that more specific diagnostic means are needed to appropriately assess the response to treatment.

Footnotes

^a JavaScript from: https://www.stat.ubc.ca/~rollin/stats/ssize/, University of California, San Francisco, CA. Last updated: October 2006

^b Rompun, Bayer, Toronto, ON, Canada

^c Torbugesic, Wyeth Animal Health, Guelph, ON, Canada

^d Lidocaine Neat, Wyeth Animal Health

^e Hema-Tek 2000, Bayer

^f Fleisch #4, Metabo, SA, Switzerland

^g Scireq Precision differential pressure transducer UT-PDP 75, Montreal, QC, Canada

^h 3L calibrated syringe, Hans Rudolph Inc, Kansas City, MO

ⁱ Scireq Data Acquisition Controller DAC 08 and flexiWare, software version 5.1, Montreal, QC, Canada

^j SCIREQ, Sigma-Aldrich, Oakfield, ON, Canada

^k Salter Labs 8900 Small Volume Jet Nebulizer, Arvin, CA

^l PulmoAid 5650C, DeVilBiss, Somerset, PA

^m Aeromask, Trudell Medical International, London, ON, Canada

ⁿ Dexamethasone 5, Vetoquinol N-A.Inc, Quebec, QC, Canada

^o Flovent HFA, GlaxoSmithKline Inc, Montreal, QC, Canada

^p AeroHippus, Trudell Medical International

^q Analytical Software Statistix 9.0 Analytical Software, Tellahassee, FL

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