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Effect of Beclomethasone Dipropionate and Dexamethasone Isonicotinate on Lung Function, Bronchoalveolar Lavage Fluid Cytology, and Transcription Factor Expression in Airways of Horses with Recurrent Airway Obstruction

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Glucocorticoid (GC) therapy is recognized to be effective for the treatment of recurrent airway obstruction (RAO) in horses. Anti-inflammatory properties of GC are thought to be mediated by suppression of inflammatory gene expression via inhibition of transcription factors such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1). The purpose of this study was to evaluate the effect of low-dose inhaled beclomethasone dipropionate and injectable dexamethasone 21-isonicotinate on clinical signs, pulmonary function, airway cytology, and activity of NF-κB and AP-1 in bronchial cells of RAO-affected horses. Seven horses with RAO were exposed to moldy hay until they developed airway obstruction on 3 separate occasions. In a crossover design, they were then treated with a placebo (injection on day 1), inhaled beclomethasone (500 μg q12h for 10 days), or dexamethasone (0.06 mg/kg, IM on day 1) and monitored for 10 days. Pulmonary function, bronchoalveolar lavage fluid cytology, and NF-κB and AP-1 activity in bronchial brushing cells were measured before (day 1) and after treatment (day 10). Treatment with beclomethasone resulted in significantly improved pulmonary function of RAO-affected horses compared with placebo and dexamethasone treatments. However, none of the treatments had an effect on bronchoalveolar lavage fluid cytology or NF-κB and AP-1 activity. These findings reveal that, in a model of severe RAO, the benefits of low-dose inhaled beclomethasone on pulmonary function are not accompanied by a decrease in airway inflammatory cells or a suppression of transcription factors NF-κB and AP-1 DNA-binding activity.

Key words: Activator protein-1; Glucocorticoids; Inflammation; Lung function; Nuclear factor-κB.

Recurrent airway obstruction (RAO) or heaves shares many features with human asthma, including distal airway inflammation, reversible airway obstruction, and bronchial hyperresponsiveness.[1,2] The equine disease is characterized by neutrophilic inflammation, which is a common feature of acute severe asthma and grain dust–induced asthma in people.[3,4] Initiation and persistence of airway inflammation is due, at least in part, to increased activity of transcription factors, such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), at the cellular level.[5] In turn, transcription factors increase the expression of inflammatory genes resulting in the production of many inflammatory mediators by various cells including bronchial epithelial cells and macrophages. In horses, Bureau et al[6] revealed that high concentrations of NF-κB activity are present in bronchial cells of RAO-affected animals exposed to moldy hay. Furthermore, the degree of lung dysfunction is highly correlated with the level of NF-κB activity in bronchial cells.

It is now well accepted that glucocorticoids (GCs) are effective in relieving airway obstruction and pulmonary inflammation in horses with RAO. Systemic GCs are relatively inexpensive and easy to administer. Administration of once daily dexamethasone to horses with RAO improves lung function by day 3 of treatment, and the effect is maximal by day 7.[7,8] A single injection of triamcinolone relieves airway obstruction for 2–3 weeks.[9] Long-acting GCs, such as triamcinolone, are more likely to cause adverse effects such as immune suppression, iatrogenic Cushing's disease, adrenal cortex suppression, and laminitis.[10] In the United States, dexamethasone 21-isonicotinate is a long-acting GC approved for use in horses as a solution, and repeated injections at 3-day intervals improve lung function of RAO-affected horses.[11] In Europe, a crystalline suspension[a] is available with pharmacological effect lasting 7–10 days according to the manufacturer's label. Therefore, dexamethasone 21-isonicotinate is less likely to induce undesirable adverse effects than triamcinolone while providing prolonged anti-inflammatory effects. To our knowledge, the efficacy of dexamethasone 21-isonicotinate suspension therapy for RAO has not been reported.

Treatment of RAO via inhalation of aerosolized GC requires a lower dose than systemic therapy while achieving an optimal drug level in the airways, thus decreasing the likelihood of adverse effects.[12] Inhaled beclomethasone dipropionate has been revealed to be effective for the treatment of RAO.[13,14] A dosage as low as 500 μg q12h, administered with a specially designed hand-held device, is sufficient to improve lung function of affected horses and minimize adrenal suppression.[15]

GCs produce their effect by induction of gene transcription (transactivation) or by repression of gene transcription (transrepression).[5] Transactivation occurs when GCs bind to intracellular GC receptors to form homodimers and interact with GC response elements in the promoter region of certain genes. Most endocrine

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and metabolic effects of GCs are thought to be mediated by transactivation. Alternatively, GCs may form monomers with GC receptors and directly bind transcription factors such as NF-κB and AP-1, thus resulting in transrepression of many inflammatory genes. This mechanism is thought to be responsible for the anti-inflammatory and immunosuppressive activity of GCs used in the treatment of human asthma. However, the effects of GCs on NF-κB and AP-1 activity in RAO-affected horses have not been reported.

Consequently, we hypothesized that treatment of RAO-affected horses with inhaled beclomethasone and systemic dexamethasone 21-isonicotinate would (1) ameliorate pulmonary dysfunction and inflammation and (2) inhibit transcription factor activity in bronchial epithelial cells.

Materials and Methods

Animals

Seven horses (6 mares, 1 gelding; 19.7 ± 6.6 years of age [mean ± SD]; 431 ± 90 kg of body weight) with a history and clinical diagnosis of RAO were used for the study. Clinical diagnosis of RAO was based on (1) apparent good health except for a history of inducible and reversible airway obstruction when exposed to moldy grass hay; (2) pleural pressure changes (ΔPplmax) $>$ 15 cmH_2O after exposure to moldy grass hay; (3) >25% of total nucleated cells were neutrophils in the bronchoalveolar lavage (BAL) fluid after moldy grass hay exposure; and (4) CBC and serum biochemistry within reference values.

Study Design

The experiment was designed as a randomized, crossover, blinded study where each of the horses received either beclomethasone dipropionate (500 μg inhaled q12 hours, from day 1 to day 10) with a hand-held delivery device,[b] dexamethasone-21 isonicotinate (0.06 mg/kg, IM once on day 1), or sterile saline solution (20 mL, IM once on day 1). All horses completed the 3 arms of the trial and remained in the same environmental conditions during the 10-day treatment period. The different routes of administration of the test and reference products were not suitable for blinding the person responsible for drug administration (BdM). To keep the other investigators blinded, a dispenser was responsible for handling the drugs either for training the person responsible for treatment with the test device (beclomethasone) or for treatment with dexamethasone or the control product.

Horses were moved from pasture to individual stalls where they were exposed to moldy grass hay (24 h/d). Clinical examinations were performed daily until horses developed typical clinical signs of respiratory disease, at which point pulmonary function testing was conducted. Moldy hay challenge was continued until RAO-affected horses exhibited ΔPplmax $>$ 15 cmH_2O (day 1). At that time, pulmonary function testing, BAL, and bronchial brushing were done. Therapy with 1 of the 3 drugs was initiated in the evening following testing. Clinical examinations, including clinical scoring, were conducted by the same investigator (LLC) on days 1, 4, 7, and 10. Nostril flaring and abdominal expiratory efforts were each graded by visual inspection of the horse on a 4-point scale from 1 = normal to 4 = severe.[14] Clinical score was obtained by adding nostril flaring and abdominal effort scores (range 2–8). Pulmonary function testing, BAL, and bronchial brushing were repeated on day 10.

At the completion of each 10-day treatment period, the animals were placed in a low-dust environment for a 4-week wash-out period. During that period, horses were kept on wood shavings and fed with silage. The same protocol was repeated during each phase of the study, except that the horses' treatments were switched according to a predetermined random treatment schedule.

Experimental Procedures

Pulmonary Function Testing. These tests were performed by the simultaneous measurement of pleural pressure changes (esophageal balloon catheter technique) and respiratory airflow (Fleisch pneumotachograph No. 4 mounted on a face mask and connected with 2 polyethylene catheters to a differential pressure transducer).[16] The raw signals were processed by a lung function computer to provide a breath-by-breath measurement of maximum transpulmonary pressure change (ΔPplmax), pulmonary resistance (R_L), and dynamic compliance (C_{dyn}). At each measurement, lung function was recorded for approximately 2 minutes, and the average values from 10 representative breaths were calculated.

Bronchoalveolar Lavage. Horses were premedicated intravenously with romifidine[c] 0.01 mg/kg. BALs were performed with a 170 cm × 12.9 mm fiberoptic endoscope wedged into a segmental right-cranial bronchus and by instillation, in situ, of 6 × 60 mL sterile saline at 37°C. The fluid was aspirated manually in 60 mL syringes and placed on ice immediately. Samples were pooled together and an aliquot collected for cytology. Specimens were prepared by cytospin and stained with May-Gruenwald. Differential cell counts were determined by examination of 200 leukocytes per slide.

Bronchial Brushings. The endoscope was kept in place after collection of the BAL and a cytology brush[d] was inserted through its biopsy channel. Collection of bronchial cells was performed by brushing 8 different places at the level of a segmental bronchus. Care was taken to avoid bleeding. The brush was retracted into its protective sheath and removed from the endoscope channel. Cells were dislodged by shaking the brush into 50-mL conical tubes filled with ice-cold RPMI 1640 medium supplemented as described for BAL cells. The harvested cell suspension was vortexed and filtered through gauze to remove mucus. The cells were then centrifuged at 800 × *g* for 5 minutes, and the pellet resuspended in LHC-8 complete medium without hydrocortisone supplemented with 10 μg mL^{-1} amphotericin B. The cells were then incubated at 37°C in a 5% CO_2–95% air mixture for different times before protein extraction. Cell density was assessed by the use of a hemocytometer, and cell viability was evaluated by propidium iodide exclusion (5 μg mL^{-1} of culture medium). Nuclear protein extractions were then performed using standard techniques.[6] The activity of the different transcription factors was determined by electrophoretic mobility shift assays.

Electrophoretic Mobility Shift Assays. The procedure for electrophoretic mobility shift assays has been described previously.[6] In summary, binding reactions were performed for 30 minutes at room temperature with 5 μg of nuclear proteins in 20 mM HEPES, pH 7.9, 10 mM KCl, 0.2 mM EDTA, 20% (vol/vol) glycerol, 1% (wt/vol) acetylated bovine serum albumin, 3 μg of poly(dI-dC), 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 100,000 cpm of ^{32}P-labeled double-stranded oligonucleotide probes. Labeled probes were purified by spin chromatography on G-25 columns. DNA-protein complexes were separated from unbound probe on 4% native polyacrylamide gels and vacuum-dried. This was followed by exposure to Fuji X-ray film at −80°C for 12 hours. The amount of specific complexes was determined by photodensitometry of the autoradiography. To confirm specificity, competition assays were performed. The sequences of the probes were as follows: NF-κB, 5′–CAA CGG CAG GGG AAT TCC

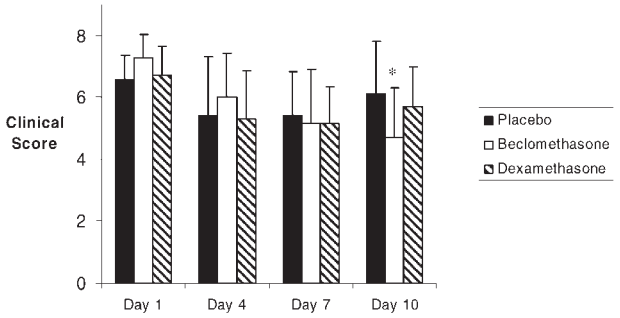

Fig 1. Clinical respiratory score of 7 horses with recurrent airway obstruction kept in moldy hay environment during treatment with placebo, beclomethasone, and dexamethasone. Placebo and dexamethasone treatments were given as a single IM injection on day 1. Beclomethasone was administered via aerosolization q12h for 10 days. *Significantly different ($P < .05$) from day 1 clinical score.

CCT CTC CTT AGG TT–3′; AP-1, 5′–CGC TTG ATG AGT CAG CCG GAA–3′.

Statistical Analysis

The effect of treatment (beclomethasone, dexamethasone, or placebo), time (day 1 or day 10) and treatment × time interaction on clinical score, pulmonary function, and BAL cytology were tested by multivariate analysis of variance.[c] When a significant time effect or time × treatment interaction was detected, differences of least-squares means were calculated for paired comparisons and P values adjusted by use of the Tukey-Kramer procedure. Comparison of transcription factor activities among groups was performed by Wilcoxon signed ranked tests. Results were expressed as mean ± standard deviation. The significance level was placed at 0.05.

Results

Clinical Evaluation

Overall, clinical scores decreased between day 1 and 10 (time effect; $P = .0028$), but no treatment effect was detected (Fig 1). Horses treated with inhaled beclomethasone exhibited a significantly lower clinical score at day 10 compared with day 1 ($P < .001$), but the score was not different from placebo-treated horses at day 10.

Pulmonary Function Testing

Values of lung function variables did not differ among the 3 groups at baseline (day 1). However, ΔPplmax was significantly reduced after 10 days of inhaled beclomethasone therapy and was lower than ΔPplmax measured 10 days after administration of a placebo ($P = .0078$; Fig 2A). Treatment with beclomethasone and dexamethasone led to a reduction of ΔPplmax of 51% and 6% respectively, in contrast to an increase of 33% after placebo treatment. There was a 39% reduction in R_L after 10 days of inhaled beclomethasone therapy although this was not statistically significant (Fig 2B, $P = .06$) whereas R_L decreased only 3% after dexamethasone treatment and increased 12% after placebo treatment. No significant time or treatment effects on C_{dyn} were detected (Fig 2C). The effect of dexamethasone on pulmonary function of

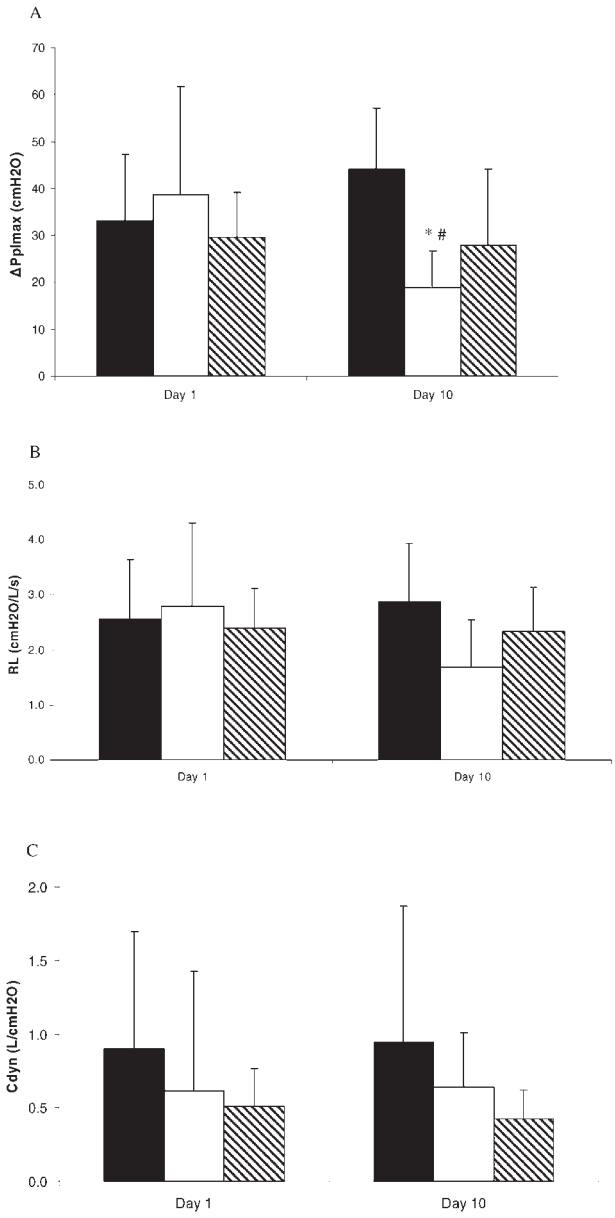

Fig 2. Pulmonary function measurements in 7 horses with recurrent airway obstruction kept in moldy hay environment during treatment with placebo, beclomethasone, and dexamethasone. Maximum transpulmonary **(A)** pressure changes (ΔPplmax), **(B)** pulmonary resistance (R_L), and **(C)** dynamic lung compliance (C_{dyn}) were measured before (day 1) and after 10 days of therapy (day 10). # Significantly different from placebo-treated horses. (See Fig 1 for legend.)

RAO-affected horses was not significantly different from placebo treatment. Treatment-period interaction for ΔPplmax was minimal and not statistically significant, indicating that the order in which horses received their treatment did not influence the outcome of the trial.

Bronchoalveolar Lavage Cytology

There was no significant difference in BAL fluid (BALF) cytology and volume of fluid recovered among

Table 1. Bronchoalveolar lavage fluid cytology of horses with recurrent airway obstruction kept in moldy hay environment during a 10-day treatment with placebo, daily inhaled beclomethasone, and a single dose of dexamethasone isonicotinate. Results as mean ± SD cells/μL.

		TNCC		Neut		Mac		Lymph		Eos		Mas	
Treatment	N	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10
Placebo	7	355 ± 363	161 ± 209	185 ± 241	66 ± 64	149 ± 125	60 ± 94	17 ± 19	30 ± 76	0 ± 0	0 ± 0	2 ± 1	2 ± 4
Beclomethasone	7	236 ± 188	566 ± 1,064	120 ± 125	311 ± 655	99 ± 76	203 ± 351	9 ± 13	25 ± 46	0 ± 1	0 ± 1	1 ± 1	7 ± 14
Dexamethasone	7	220 ± 298	336 ± 422	178 ± 236	287 ± 360	57 ± 70	85 ± 77	9 ± 15	6 ± 7	1 ± 1	0 ± 0	2 ± 2	2 ± 2

TNCC, total nucleated cell count; Neut, neutrophils; MAC, alveolar macrophage; Lymph, lymphocytes; EOS, eosinophils; MAS, mast cells.

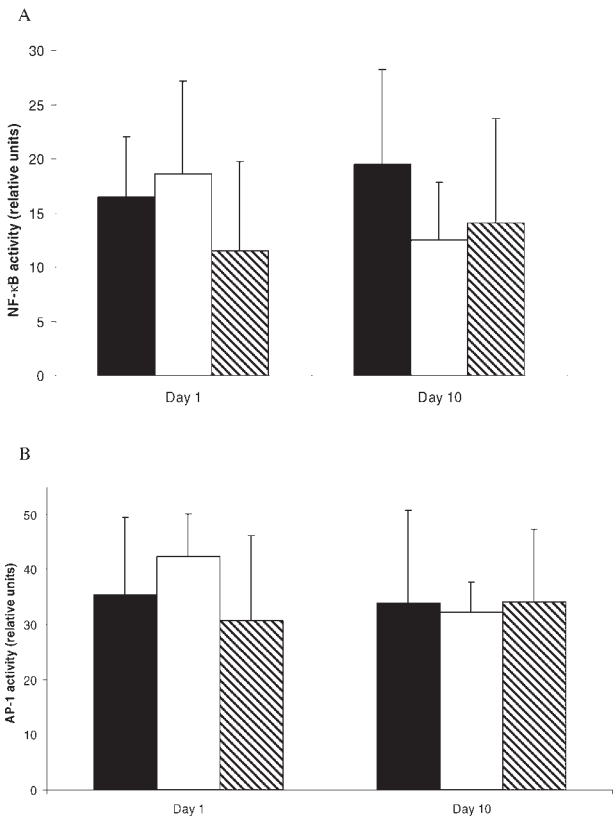

Fig 3. DNA-binding activity of **(A)** NF-κB and **(B)** AP-1 in bronchial brushings collected from 7 horses with recurrent airway obstruction kept in moldy hay environment during a 10-day treatment with placebo, beclomethasone, and dexamethasone.

treatment groups at baseline (day1). There was no significant effect of treatment on BALF cytology (Table 1).

Transcription Factor Activity

Measurements of NF-κB and AP-1 activity in cells collected by bronchial brushings did not differ among the 3 groups at baseline (day 1). No significant treatment effects on transcription factors activity were detected (Fig 3). AP-1 activity decreased 24% after 10 days of treatment with beclomethasone, whereas AP-1 activity decreased only 4% after placebo treatment and increased 11% after dexamethasone treatment; however, this time effect of the beclomethasone treatment did not reach statistical significance ($P = .066$). Also, AP-1 activity on day 10 was not different among the 3 treatment groups.

Discussion

A 10-day course of inhaled beclomethasone improved pulmonary function of RAO-affected horses maintained in a moldy hay environment as compared with placebo treatment. However, no treatment benefit was detected on indicators of airway inflammation, such as BALF cytology or transcription factor activity, in bronchial brushing cells. Administration of a single dose of dexamethasone 21-isonicotinate did not result in any

detectable benefit in RAO horses' clinical signs, pulmonary function, or airway inflammation 10 days later.

The dose of aerosolized beclomethasone used in this study (500 μg q12h) is the lowest dose that has been revealed to significantly improve clinical signs and lung function in RAO-affected horses maintained in a moldy hay environment.[15] Our findings confirm that a 10-day treatment with low-dose beclomethasone results in improved pulmonary function. The clinical benefit was not detectable when using subjective criteria such as nostril flaring and abdominal expiratory efforts. The difference in clinical and functional response between the 2 studies may have resulted from differences in allergen challenge, disease severity, study design, or drug delivery. Rush et al[15] reported that ΔPplmax and R_L decreased by 15–20% in placebo-treated horses after 7-day confinement in a moldy hay environment. Our study found that ΔPplmax and R_L increased by 33% and 12%, respectively, after 10 days in a moldy hay environment. This increased degree of airway obstruction may have resulted from a more severe allergenic challenge, which could have led to a decreased treatment efficacy. Alternatively, our study used an injectable placebo whereas Rush et al[15] administered an aerosolized placebo treatment via a device inserted in the horse's nose, which by itself may cause bronchodilation.[17] Comparison of clinical score and pulmonary function tests at baseline reveals that disease severity was comparable between the groups of RAO horses used in both studies. Also, similar crossover designs and clinical scoring systems were used in the 2 studies. However, correlations between clinical scores and indices of pulmonary function are weak, and large differences in pulmonary function are required before changes in clinical signs are noticed.[18]

The lack of a statistically significant effect of dexamethasone 21-isonicotinate observed in this study may have been because of an inappropriate dose or regimen. The soluble form of dexamethasone 21-isonicotinate (Voren® solution) is therapeutically equivalent to dexamethasone, and IM administration of its soluble form (Voren® solution) results in adrenal suppression that may last up to 4 days.[19] Horses in our study received the recommended dose of 0.06 mg/kg IM of dexamethasone 21-isonicotinate on day 1 of the trial. Previous studies have revealed that daily administration of 0.04 mg/kg of dexamethasone to RAO-affected horses maintained in moldy hay environments results in significant improvement in lung function starting on the 3rd day of administration with a maximum benefit by the end of 2 weeks of therapy.[20,21] If horses are maintained in the moldy hay environment, pulmonary function may return to pretreatment levels as early as 6 days after discontinuation of treatment.[20] This could explain the lack of effect of dexamethasone 21-isonicotinate on pulmonary function 10 days after a single injection under these experimental conditions. In this study, horses received a microcrystalline insoluble form of dexamethasone 21-isonicotinate (Voren® Depot™) that results in peak dexamethasone concentration 24 hours after administration and sustained serum concentrations as long as 14 days.[22] Serum dexamethasone concentrations between 1 and 14 days after administration correspond to concentrations obtained 24 hours after administration of the same dose of dexamethasone alcohol. Therefore, the dose of dexamethasone 21-isonicotinate used in our study was probably too low to benefit pulmonary function of RAO-affected horses under these experimental conditions.

Ten-day therapy with 500 μg of aerosolized beclomethasone q12h did not affect BALF cytology of RAO-affected horses. The fact that pulmonary function improved after beclomethasone therapy, but BALF cytology was unaffected suggests a lack of correlation between inflammatory cells in epithelial lining fluid and the degree of airway obstruction. Increase in ΔPplmax and pulmonary neutrophilia occur concomitantly within 4–5 hours of exposure of RAO-affected horses to moldy hay; however, some horses may develop pulmonary dysfunction without neutrophil accumulation in the lungs.[23] After RAO-affected horses are placed in a low-dust environment, pulmonary function normalizes; however, neutrophil count in BALF may remain high for a prolonged time.[24] A previous study revealed that 1,320 μg of aerosolized beclomethasone q12h for a week resulted in decreased BALF neutrophil counts between 3 and 7 days after treatment initiation.[25] However, some RAO-affected horses treated with aerosolized or systemic GCs may improve clinically and functionally without detectable changes in BALF neutrophil counts.[9,11,26] This apparent discrepancy between airway inflammatory cells and pulmonary function may result from the factors involved in balancing recruitment and removal of inflammatory cells in the lung. In human asthma, recruitment of leukocytes to the lungs and their activation are controlled by chemotactic cytokine released by airway epithelial cells and macrophages.[27] Apoptosis allows safe removal of inflammatory cells, such as eosinophils, and may be inhibited by various inflammatory mediators (thus prolonging the cell's life span) or stimulated by GCs (thus shortening the cell's life span). Lung granulocytes from RAO-affected horses fed moldy hay also demonstrate delayed apoptosis, thereby contributing to the maintenance of airway neutrophilia in horses that are kept in an allergenic environment.[28] However, GCs inhibit apoptosis of human neutrophils. Whether or not GCs have similar effect on equine neutrophils is unknown.

Airway inflammation is characterized by recruitment of inflammatory cells and increased production of inflammatory mediators. Regulation of inflammatory gene expression is controlled by transcription factors, which appear to play a key role in the pathophysiology of inflammatory lung diseases such as asthma.[5] Rodent models of asthma suggest that transcription factors, such as NF-κB and AP-1, are central to the pathophysiology of pulmonary inflammation.[29–33] Both transcription factors appear to be up-regulated in the airways of asthmatics.[34,35] Similarly, increased NF-κB has been demonstrated in the airways of RAO-affected horses exposed to moldy hay.[6] In many mammalian cells,

NF-κB complexes are mainly composed of p65-p50 heterodimers; however, in equine airways NF-κB complexes are mainly composed of p65-p65 homodimers.[6,36] In agreement with previous findings, we observed that bronchial cells of RAO-affected horses exhibited high DNA-binding activity for NF-κB (p65). To our knowledge, this is the 1st reported measurement of AP-1 activity in bronchial cells of RAO-affected horses. Activator protein-1 is composed of homo- or heterodimers of the Fos and Jun families of transcription factors.[5] As for NF-κB, AP-1 binding sites are present in the promoter region of many genes coding for proinflammatory mediators. The main role of these transcription factors appears to be the conversion of environmental signals on airway cell surfaces into increased expression of inflammatory genes.

Three consecutive exposures of RAO-affected horses to moldy hay environments resulted in comparable levels of pulmonary dysfunction and alteration in BALF cytology and bronchial cell NF-κB and AP-1 activity. Horses treated with a placebo or a single injection of dexamethasone isonicotinate and kept in a dusty environment exhibited similar level of pulmonary dysfunction, alteration in BALF cytology, and bronchial cell transcription factor activity 10 days later. These findings suggest that the moldy hay challenge was repeatable and provided a maximum antigenic stimulation throughout the 3 arms of the crossover trial.

The major anti-inflammatory effects of GCs appear to be related to direct binding of glucocorticoid receptor-GC complexes to NF-κB and AP-1, thereby preventing them from binding to DNA and inducing transcription of proinflammatory genes.[5,37] In our study, administration of GCs did not result in significant changes in bronchial cell NF-κB and AP-1 DNA-binding activity or BALF cytology, even though pulmonary function improved in horses treated with inhaled beclomethasone. Several hypotheses may be formulated to explain this apparently contradictory observation: (1) the effects of GCs on pulmonary function may be mediated via NF-κB– and AP-1–independent pathways, (2) the variability in nuclear factor measurements did not allow detection of a GC effect, or (3) the dose of GCs was sufficient to improve pulmonary function but too low to suppress airway inflammation. The finding that administration of inhaled GCs to human asthmatics results in decreased NF-κB and AP-1 activity in airway epithelium would contradict the 1st hypothesis.[38–40] Nevertheless, one study revealed no effect of inhaled GCs on NF-κB activity in the airway epithelial cells of asthmatic patients despite improvement in pulmonary function.[41] Furthermore, the number of epithelial cells containing p65 increased after treatment with an inhaled GC (fluticasone propionate). Inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor–α (TNF-α) activate NF-κB and AP-1 through a variety of signaling pathways, resulting in chromatin remodeling (DNA unwinding) that allows DNA-binding of transcription factors and RNA polymerase II complexes and initiation of gene transcription.[42,43] Activated GC receptors block gene transcription mainly by directly binding to NF-κB and AP-1; however, several different steps of this signaling pathway may be inhibited.[43] Interestingly, a low dose of GCs reverses the unwinding of DNA, thereby switching off inflammatory gene expression.[44] This anti-inflammatory effect of GCs does not interfere with NF-κB and AP-1 DNA-binding activity. In RAO-affected horses exposed to moldy hay, inflammatory mediators such as IL-1β and TNF-α continuously activate NF-κB, thereby maintaining a high level of transcription factor activity (ie, p65) in the airways.[45] Therefore, GC inhibition of DNA unwinding could suppress inflammatory gene expression while allowing a high level of activated NF-κB and AP-1 to be maintained in cells. This possibility could be assessed further by measuring the expression of inflammatory cytokines, such as IL-4, IL-5, IL-8, and ICAM-1 in epithelial cells, which was not performed in this study.

The 2nd possible explanation for the discrepancy between transcription factor activity and pulmonary function is that the large variability in transcription factor measurements masked the effect of the GCs. In this study, we used 7 horses. However, based on observed within-horse standard deviation, a minimum of 12 horses would have been needed to detect a treatment effect with >80% power in a paired analysis at $P < .05$. Finally, higher doses of inhaled beclomethasone (1,320 μg, q12h) have been revealed to significantly improve pulmonary function and decrease BALF neutrophils.[25] The dose used in our study (500 μg, q12h) may have been sufficient to improve pulmonary function according to mechanisms described above but insufficient to result in a reduction in BALF neutrophils. This explanation would be consistent with a report of human asthmatics that exhibited improvement in pulmonary function after treatment with inhaled fluticasone but revealed no change in BALF neutrophils or NF-κB activity in epithelial cells.[41]

In conclusion, 10 days after administration of a single dose of long-acting dexamethasone to RAO-affected horses maintained in an experimental moldy hay environment, no statistically significant improvement in pulmonary function nor reduction in inflammatory markers was detectable. A 10-day course of low-dose inhaled beclomethasone (20 treatments) resulted in improved pulmonary function but without detectable changes in airway inflammatory cell numbers or NF-κB and AP-1 activity. The reason for this dissociation between the level of airway obstruction and inflammatory markers in response to GC therapy is unknown and will require further investigation.

Footnotes

[a] Voren Depot, Boehringer Ingelheim Vetmedica GmbH, Ingelheim/Rhein, Germany

[b] 3M-Boehringer Ingelheim Vetmedica GmbH, Ingelheim/Rhein, Germany

[c] Sedivet, Boehringer Ingelheim Vetmedica GmbH, Ingelheim/Rhein, Germany

[d] Cytology brush, Cook Veterinary Products, Eight Mile Plains, Australia

[e] General linear model statistical software, SAS Institute Inc, Cary, NC.

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