



# Bioavailability and tolerability of nebulised dexamethasone sodium phosphate in adult horses

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## Summary

**Background:** Nebulisation of the injectable dexamethasone sodium phosphate (DSP) would offer an inexpensive way of delivering a potent corticosteroid directly to the lungs of horses with asthma. However, this approach would be advantageous only if systemic absorption is minimal and if the preservatives present in the formulation do not induce airway inflammation.

**Objective:** To investigate the bioavailability of nebulised DSP and determine whether it induces airway inflammation or hypothalamic-pituitary-adrenal (HPA) axis suppression in healthy adult horses.

**Study design:** Randomised crossover experiment.

**Methods:** Dexamethasone sodium phosphate was administered to six healthy adult horses at a dose of 5 mg q. 24 h for 5 days via nebulised, or intravenous (i.v.) routes. Plasma dexamethasone concentrations were measured by UPLC/MS-MS to calculate bioavailability. Cytological examination of bronchoalveolar fluid was performed at baseline and after the last dose of DSP. A validated chemiluminescent immunoassay was used to measure basal serum cortisol concentrations.

**Results:** After nebulisation to adult horses, dexamethasone had a mean ( $\pm$ s.d.) maximum plasma concentration of  $0.774 \pm 0.215$  ng/mL and systemic bioavailability of  $4.3 \pm 1.2\%$ . Regardless of route of administration, there was a significant decrease in the percentage of neutrophils in bronchoalveolar lavage fluid over time. During i.v. administration, basal serum cortisol concentration decreased significantly from baseline to Day 3 and remained low on Day 5. In contrast, basal serum cortisol concentration did not change significantly during administration via nebulisation.

**Main limitations:** Small sample size and short period of drug administration.

**Conclusions:** Dexamethasone sodium phosphate administered via nebulisation had minimal systemic bioavailability and did not induce lower airway inflammation or HPA axis suppression in healthy horses.

**Keywords:** horse; nebulised; corticosteroids; dexamethasone; adult

## Introduction

Equine asthma is the new terminology used to describe horses with chronic nonseptic lower airway inflammation [1,2]. Two phenotypes of equine asthma have long been recognised: 1) heaves (also known as recurrent airway obstruction) and 2) inflammatory airway disease (IAD). Decreased exposure to dust and offending allergens is essential for the long-term control of equine asthma. However, for many severely affected horses environmental management alone is not sufficient to prevent airway inflammation and medical therapy is needed.

Corticosteroids are the most effective form of medical therapy in horses with asthma due to their potent anti-inflammatory effects [1,3]. Systemic corticosteroids, such as dexamethasone, are inexpensive and very effective for short-term treatment during acute exacerbation of clinical signs [4]. However, many horses with heaves require long-term anti-inflammatory therapy with corticosteroids to control the inflammatory response and prevent recurrent episodes of respiratory distress. Unfortunately, long-term use of systemic corticosteroids has been associated with, or suspected to result in, potentially life threatening adverse effects such as laminitis, altered bone metabolism [5], systemic infections [6], decreased antibody production in response to vaccination [7] and hypothalamic-pituitary-adrenal (HPA) axis suppression [8].

Availability of inhaled corticosteroid therapy has revolutionised the treatment of asthma in people by allowing long-term control of symptoms, while minimising adverse effects associated with systemic formulations [9]. Inhaled corticosteroids approved for the treatment of asthma in people, such as fluticasone propionate, or beclomethasone are also effective in horses [10–12] and apparently safe for long-term administration [13], but they are cost prohibitive for many horse owners. Nebulisation of the injectable formulation of dexamethasone offers an inexpensive means of delivering a potent corticosteroid directly to the lungs. Nebulised

dexamethasone is rarely used in people with asthma because of availability of many other inhaled corticosteroids specifically labeled for that purpose. Nevertheless, in two small clinical trials, nebulised dexamethasone sodium phosphate (DSP) was as effective as oral prednisone for the treatment of asthmatic children [14,15].

Nebulised DSP is inexpensive and has the potential to be effective for the treatment of equine asthma. However, this approach would be advantageous only if systemic bioavailability is low, thereby limiting the potential for systemic adverse effects such as HPA axis suppression. Aerosol use of drug formulations intended for systemic use can lead to airway inflammation due to various preservatives, additives and inappropriate pH or osmolality ranges. Therefore, it is essential to ensure that nebulised DSP does not induce airway inflammation in healthy horses before it can be recommended for use in horses with asthma. The objectives of this study were to calculate the systemic bioavailability of nebulised DSP and determine if nebulised DSP induces airway inflammation and HPA axis suppression in healthy horses. It was hypothesised that nebulised DSP has low bioavailability in healthy horses and does not induce airway inflammation after 5 days of administration.

## Materials and methods

### Animals and experimental design

Three Quarter Horse and 3 Warmblood horses between 6 and 15 years of age (one mare and five geldings; 521–612 kg) were used in this study. Horses were determined to be healthy prior to the beginning of the study based on a complete physical examination including auscultation of the lungs using a rebreathing bag, normal complete blood count and biochemistry profile and negative screening for pituitary pars

intermedia dysfunction as determined by plasma ACTH concentration <35 pg/mL using a validated chemoluminescent assay (Immulite)<sup>a</sup> on a sample collected during the summer months (early June in the Southeastern United States).

Horses were brought in from pasture at least 24 h prior to the beginning of the study and kept in individual stalls with ad libitum access to hay and water throughout the experimental period. Dexamethasone sodium phosphate (Dexaject SP)<sup>b</sup> was administered at a dose of 5 mg q. 24 h for 5 days via nebulised, or intravenous (i.v.) routes using a randomised crossover design, with a washout period of 3 weeks between each administration period. This washout period was deemed to be adequate because a single i.v. administration of DSP at the much higher dose of 0.05 mg/kg bwt inhibits endogenous cortisol suppression for only 4–5 days [16]. The dose of DSP used in this study (5 mg) was selected based on the fact that nebulised DSP at a dose of 1 mg was shown to be effective in people with asthma [14] and horses have an alveolar surface area that is approximately five times larger than that of people [17,18]. In addition, this dose is similar to daily doses of inhaled fluticasone (4–6 mg) or beclomethasone (7.5 mg) shown to be effective in horses [10–12]. For both groups, DSP was diluted 1:1 in 0.9% NaCl and administered i.v. by direct jugular venipuncture, or using a commercially available nebuliser for horses (Flexineb).<sup>c</sup> Particle size of nebulised DSP was verified using laser diffraction (Spraytec).<sup>d</sup>

### Systemic bioavailability of nebulised DSP

On the first day of sampling for each route of administration, blood for the measurement of dexamethasone concentrations was collected via a catheter placed in a jugular vein before administration of the first dose of DSP (time 0) and then 5, 10, 20, 30, 45 min, as well as 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after drug administration. The concentration of dexamethasone in plasma was determined by ultra-performance liquid chromatography with tandem mass spectrometry (UPLC/MS-MS) as detailed in Supplementary Item 1. The lower limit of detection and quantification was set as the lowest concentrations that was consistently linear on the calibration curve (0.5 ng/mL). Accuracy and precision of the assay was tested at concentrations of 50, 5 and 0.1 ng/mL. Accuracy was within  $6.07 \pm 4.8\%$  of the true value and precision within  $2.37 \pm 1.71\%$  of the mean.

For each animal, plasma dexamethasone concentration vs. time data was analysed based on noncompartmental pharmacokinetics using commercially available software.<sup>e</sup> Maximum plasma concentration ( $C_{max}$ ) and time to achieve maximum plasma concentration after nebulisation ( $T_{max}$ ) was obtained directly from the data. The rate constant of the terminal phase ( $\lambda_z$ ) after i.v. administration was determined by linear regression of the logarithmic plasma concentration vs. time curve using a minimum of three data points. Half-life of the terminal phase ( $t_{1/2z}$ ) was calculated as  $\ln 2$  divided by  $\lambda_z$ . The area under the concentration-time curve (AUC) and the area under the first moment of the concentration-time curve (AUMC) was calculated using the trapezoidal rule, with extrapolation to infinity using  $C_{24h}/\lambda_z$ , where  $C_{24h}$  is the plasma activity at the 24 h sampling time. Mean residence time (MRT) was calculated as:  $AUMC/AUC$ . Bioavailability of nebulised DSP was estimated as  $AUC_{NEB}/AUC_{IV}$ , where  $AUC_{NEB}$  and  $AUC_{IV}$  are the AUC after nebulisation and i.v. administration, respectively. Apparent volume of distribution based on the AUC ( $V_{darea}$ ) was calculated as:  $i.v. \text{ dose} / AUC \cdot \lambda_z$  and systemic clearance (CL) calculated from:  $i.v. \text{ dose} / AUC$ .

### Effect of DSP on HPA axis function

For each route of administration, ACTH stimulation testing was performed prior to administration of DSP on Day 1 and before administration of the last dose of DSP on Day 5. For each ACTH stimulation test, blood was collected for measurement of basal serum cortisol concentrations at 06.00 h, immediately before administration of 1 µg/kg of synthetic ACTH (cosyntropin; Cortrosyn)<sup>f</sup> as a rapid i.v. bolus. Blood was collected 90 min later for assessment of the cortisol response to cosyntropin. The increase in cortisol in response to cosyntropin was calculated by subtracting the basal cortisol concentration from the cortisol concentration 90 min after administration of cosyntropin. Blood for the measurement of basal serum cortisol concentration was also collected during the 5 days DSP

administration period, at 06.00 h just prior to DSP administration on Day 3. Serum cortisol concentrations were measured using a validated chemiluminescent immunoassay.<sup>a</sup>

### Effect of nebulised DSP on airway cytology

For each route of administration, a bronchoalveolar lavage (BAL) was performed prior to administration of DSP on Day 1 (baseline) and again 2 h after the last dose of DSP on Day 5. Horses were sedated with xylazine (0.5 mg/kg bwt i.v.) and butorphanol (0.05 mg/kg bwt i.v.) for BAL fluid collection. A 10 mm in diameter, 2.4 m BAL catheter<sup>g</sup> was passed via nasal approach until wedged into a bronchus. The lavage solution consisted of four aliquots of 50 mL physiological saline (0.9% NaCl) solution infused and aspirated immediately. Total nucleated cell count in BAL fluid was determined using an automated cell counter (cellometer Auto T4).<sup>h</sup> Slides of the BAL fluid were prepared by cytocentrifugation and air dried slides stained using Wright-Giemsa. Differential count was made by examining 400 cells.

### Data analysis

A priori sample size calculations for differences between two dependent means revealed that four horses per group would be required to detect a 5% increase in the percentage of neutrophils in the BAL fluid of horses nebulised with DSP as being significantly different from that of horses receiving i.v. DSP with a correlation between groups of 0.5,  $\alpha$  of 0.05 and statistical power of 80%. These calculations were based on the percentage of neutrophils of  $5.9 \pm 1\%$  in 86 healthy adult horses [19]. Similarly, sample size calculations for differences between two dependent means revealed that five horses per group would be required to detect a 60% decrease in basal cortisol concentration in horses treated with i.v. DSP as being significantly different from concentrations observed in the same horses receiving nebulised DSP with a correlation between groups of 0.5,  $\alpha$  of 0.05 and statistical power of 80%. These calculations were based on basal cortisol concentrations measured in 15 healthy horses ( $135.2 \pm 63.5$  nmol/l) during the same season in the same geographic location [20]. We enrolled 6 horses to allow for variation in observed values from hypothetical values used for calculations.

Normality of the data was assessed based on examination of histograms and normal Q-Q plots of the residuals and using Shapiro-Wilk's test. Constant variance of the data was assessed by plotting residuals against predicted values. Data were analysed using linear mixed-effects modeling with horse modeled as a random effect and route of administration (nebulised vs. i.v.) and day of the study modeled as fixed nominal effects. In preliminary analyses, order of treatment administration was also considered, but was not significant and not retained in the final model. Interaction terms (three- and two-way) were evaluated. Model fit was assessed using Akaike's information criterion values. For each dependent variable, a random intercept model provided the best fit. A diagonal covariance structure of the residuals improved model fit for the increase in cortisol data. Addition of various covariance structures of the residuals did not improve model fit for other dependent variables. Restricted maximum likelihood estimation was used for the final models. Comparisons between the percentages of lymphocytes and macrophages after treatment with either i.v. or nebulised DSP were also performed using linear mixed-effects modeling using baseline (i.e. prior to drug administration) percentages as covariates to adjust for differing baseline values. All multiple pairwise comparisons were performed using the method of Holm-Sidak to control for family-wise type 1 error rates. For all analyses, an adjusted value of  $P \leq 0.05$  was considered statistically significant.

### Results

Adverse effects were not noted after administration of i.v. or nebulised DSP. The median particle size of nebulised DSP was 3.5 µm with 69% of the particles being <5 µm and 91% being <10 µm. In one horse, a portion of the i.v. DSP was inadvertently administered perivenous. The data from this horse was not used to derive pharmacokinetic variables after i.v. administration, or for calculation of bioavailability. The plasma concentration of dexamethasone vs. time profiles after i.v. and nebulised

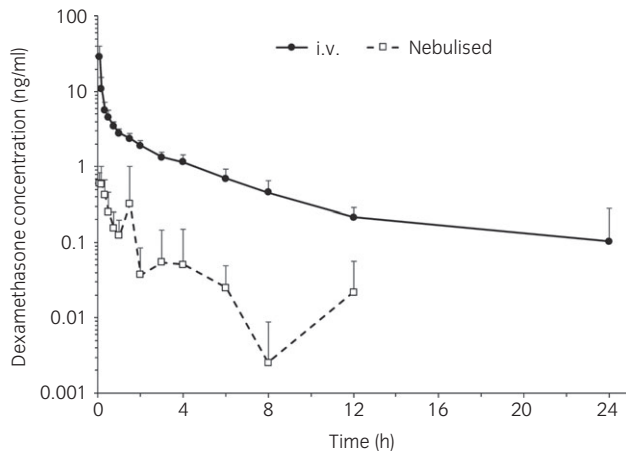


Fig 1: Mean ( $\pm$ s.d.) plasma dexamethasone concentration after administration of 5 mg of dexamethasone sodium phosphate by i.v. administration ( $n = 5$ ), or nebulisation ( $n = 6$ ).

**TABLE 1: Plasma pharmacokinetic variables (mean  $\pm$  s.d. unless otherwise specified) after i.v. or nebulised administration of 5 mg of dexamethasone sodium phosphate to five healthy adult horses**

Variable	Intravenous	Nebulised
$\lambda_{z2}$ (h)	0.194 $\pm$ 0.032	NA
$t_{1/2z}$ (h)	3.93 $\pm$ 0.55	NA
AUC <sub>0-24 h</sub> (ng·h/mL)	19.1 $\pm$ 1.52	0.885 $\pm$ 0.505
AUC <sub>0-∞</sub> (ng·h/mL)	20.1 $\pm$ 1.6	NA
MRT (h)	4.70 $\pm$ 0.983	NA
Vd <sub>area</sub> (L/kg)	2.55 $\pm$ 0.335	NA
CL (mL/h/kg)	462.9 $\pm$ 40.38	NA
C <sub>max</sub> (ng/mL)	NA	0.774 $\pm$ 0.215
T <sub>max</sub> (h)*	NA	0.25 (0.08–1.5)
F (%)	NA	4.3 $\pm$ 1.2

\*Median and range.

NA, not applicable;  $\lambda_{z2}$ , rate constant of the terminal phase;  $t_{1/2z}$ , half-life of the terminal phase; AUC<sub>0-24 h</sub>, area under the plasma concentration vs. time curve from time 0 to 24 h; AUC<sub>0-∞</sub>, area under the plasma concentration vs. time curve extrapolated to infinity; MRT, mean residence time; Vd<sub>area</sub>, apparent volume of distribution based on AUC; CL, systemic clearance; C<sub>max</sub>, maximum plasma concentration (observed) after the first dose; T<sub>max</sub>, time to maximum plasma concentration (observed) after the first dose; F, bioavailability.

administration are displayed in Figure 1. Mean ( $\pm$ s.d.) elimination half-life, apparent Vd and systemic clearance after i.v. administration were 3.93  $\pm$  0.55 h, 2.55  $\pm$  0.335 L/kg and 462.9  $\pm$  40.38 mL/h/kg, respectively (Table 1). Bioavailability of nebulised DSP sodium phosphate was 4.3  $\pm$  1.2% (Table 1).

There was a significant effect of route of administration ( $P < 0.001$ ), day ( $P < 0.001$ ) and a significant interaction between route and day ( $P < 0.001$ ) on basal serum cortisol concentrations. Basal cortisol concentrations at baseline were similar for both routes of administration (Fig 2). During i.v. administration, basal serum cortisol concentration decreased significantly from Day 1 (baseline) to Days 3 and 5. Basal serum cortisol concentration did not change significantly during administration via nebulisation (Fig 2). Basal cortisol concentrations were significantly ( $P < 0.001$ ) lower after i.v. administration than after nebulisation on Days 3 and 5. The effects of route of administration ( $P = 0.1$ ), day ( $P = 0.7$ ) and interactions between route and day on the increase in cortisol after administration of cosyntropin were not statistically significant (Fig 3).

The effects of route of administration (i.v. vs. nebulised), day of the study (baseline vs. Day 5) and interaction term between route and day

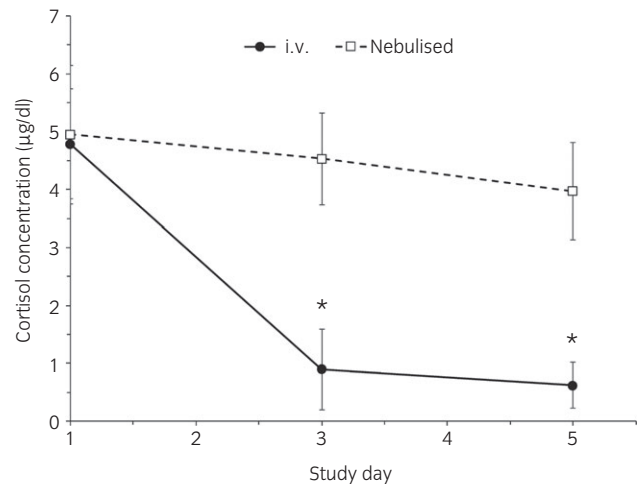


Fig 2: Mean ( $\pm$ s.d.) basal serum cortisol concentrations in six healthy adult horses before (Day 1) and 24 h after administration of two (Day 3) or four daily doses (Day 5) of dexamethasone sodium phosphate (5 mg) administered i.v., or by nebulisation. \*Significantly different from baseline value and from nebulised group at the same time point ( $P < 0.001$ ).

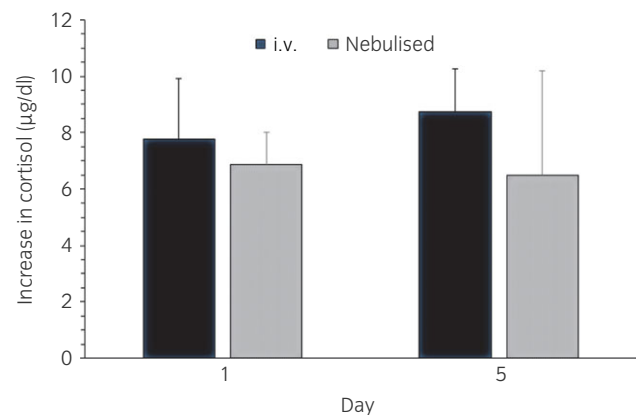


Fig 3: Mean ( $\pm$ s.d.) increase in serum cortisol concentrations 90 min after i.v. administration of 1  $\mu$ g/kg of cosyntropin in six healthy adult horses before (Day 1) and 24 h after administration of four daily doses (Day 5) of dexamethasone sodium phosphate (5 mg) administered i.v., or by nebulisation.

were not statistically significant for total nucleated cell counts and percentage of eosinophils in BAL fluid (Table 2). There was a significant effect of day ( $P < 0.001$ ) on the percentage of neutrophils but the effect of route ( $P = 0.6$ ) and interaction between day and routes ( $P = 0.3$ ) were not statistically significant. Regardless of route of administration, mean percentage of neutrophils in BAL fluid decreased significantly from baseline to Day 5 (Table 2). There were significant interactions between route and day for the percentage of lymphocytes ( $P = 0.05$ ) and macrophages ( $P = 0.03$ ). The baseline percentage of lymphocytes was significantly higher for the i.v. than for the nebulised route. In addition, there was a significant ( $P = 0.006$ ) decrease in the percentage of lymphocytes between baseline and Day 5 only in horses receiving DSP IV (Table 2). Conversely, the baseline percentage of macrophages was significantly higher for the nebulised than for the i.v. route. In addition, there was a significant ( $P = 0.006$ ) increase in the percentage of macrophages between baseline and Day 5 only in horses receiving DSP IV (Table 2). After adjusting for differing baseline percentages, the percentages of lymphocytes and macrophages on Day 5 were not significantly different between the two routes of administration.

**TABLE 2: Mean ( $\pm$ s.d.) total nucleated cell count and differential in the BAL fluid of six healthy adult horses before administration (baseline) and after five consecutive days of therapy with 5 mg of dexamethasone sodium phosphate administered i.v., or by nebulisation**

Variable	Time	Route	
		i.v.	Nebulised
Nucleated cells ( $\times 10^3/\mu\text{L}$ )	Baseline	4.21 $\pm$ 0.83	5.22 $\pm$ 2.76
	Day 5	5.20 $\pm$ 2.12	5.80 $\pm$ 2.65
Neutrophils (%)	Baseline	15.8 $\pm$ 6.4	13.5 $\pm$ 4.7
	Day 5	7.5 $\pm$ 4.6*	7.9 $\pm$ 3.5*
Lymphocytes (%)	Baseline	60.5 $\pm$ 4.4 <sup>†</sup>	49.2 $\pm$ 6.4
	Day 5	50.0 $\pm$ 6.5 <sup>‡</sup>	48.5 $\pm$ 6.3
Macrophages (%)	Baseline	22.5 $\pm$ 3.5 <sup>†</sup>	36.2 $\pm$ 7.0
	Day 5	41.5 $\pm$ 9.5 <sup>‡</sup>	42.7 $\pm$ 7.3
Eosinophils (%)	Baseline	1.1 $\pm$ 2.3	1.2 $\pm$ 2.3
	Day 5	1.1 $\pm$ 1.9	1.0 $\pm$ 1.8

\*Significantly ( $P \leq 0.05$ ) lower than the percentage of neutrophils at baseline.

<sup>†</sup>Significantly different ( $P \leq 0.05$ ) from the baseline percentage for the nebulised route.

<sup>‡</sup>Significantly different ( $P \leq 0.05$ ) from the baseline percentage for the i.v. route.

## Discussion

The goal of inhaled corticosteroid therapy is to produce long-lasting therapeutic effects in the lungs while minimising systemic bioavailability associated with oral or parenteral administration. The present study documented that the systemic bioavailability of nebulised DSP after a single administration is very low thereby minimising the risks associated with high systemic drug concentrations. In addition, daily administration of nebulised DSP did not significantly decrease basal or ACTH-stimulated cortisol concentration even after repeated daily administrations.

The mean systemic bioavailability of nebulised DSP in this study (mean = 4.2%) was considerably lower than that reported after nebulisation of the same formulation to healthy people (mean = 25%) [21]. However, bioavailability of nebulised DSP was highly variable in this study (range = 0.5–8.2%) as also reported in people (range = 8.7–46.5%) [21]. Blood corticosteroid concentrations measured after administration via inhalation represent the sum of pulmonary and orally absorbed fractions. Given that the bioavailability of oral dexamethasone in horses ranges between 23 and 88% [22,23] and that the fraction of the nebulised dose swallowed is unknown, it was not possible to determine the true pulmonary bioavailability of DSP in the present study. Fluticasone propionate has very low (0.6–1.7%) oral bioavailability in people and pulmonary bioavailability in healthy volunteers is estimated to range between 13.6 and 18% [24]. Although oral or pulmonary bioavailability of fluticasone propionate have not been studied in horses, plasma concentrations can be detected for at least 72 h after administration with a metered dose inhaler [25] indicating that the drug is partially absorbed in the respiratory and/or gastrointestinal tract. The very low bioavailability of DSP observed in this study could be the result of low pulmonary absorption of DSP in horses. Alternatively, it is possible that the drug deposition in the upper airways was high with minimal delivery to the lungs. This is unlikely given that particle size was optimal for delivery to the lower airways (median = 3.5  $\mu\text{m}$  with 91% of particles being  $< 10 \mu\text{m}$ ). In people, particles  $> 10 \mu\text{m}$  are typically filtered in the nose and nasopharynx, particles of 5–10  $\mu\text{m}$  generally reach the larger airways, and particles of 1–5  $\mu\text{m}$  reach the periphery of the lungs [26]. In addition, prior studies using the same commercially available nebuliser have documented adequate delivery of various drugs to the respiratory tract with high drug pulmonary epithelial lining fluid concentrations [27,28]. Additional studies will be required to determine the pulmonary disposition of nebulised DSP in horses and assess the effect of the method of aerosol generation and delivery on the deposition of DSP in the airways.

Nebulised DSP was as effective as oral prednisone for the treatment of asthmatic children in two small clinical trials [14,15]. In another study, nebulised DSP was found to be superior to standard therapy with metered dose or dry powder inhalers in a small number of asthmatic adult patients [29]. Nevertheless, nebulised DSP is rarely used in people with asthma due to availability of many other inhaled corticosteroids specifically developed for that purpose. In addition, administration of nebulised DSP to asthmatic children inhibited the HPA axis as much as systemic therapy [14] suggesting that nebulisation might not avoid the potential adverse effects of systemic administration. In the present study, nebulised DSP did not result in a statistically significant reduction in basal cortisol secretion or cortisol responses to ACTH even after four daily administrations which is consistent with the low systemic bioavailability observed. However, these findings must be interpreted in the context of the small sample size and relatively short period of drug administration. In contrast, basal cortisol secretion was rapidly and profoundly suppressed after i.v. administration, although adrenal cortisol responses to supraphysiological concentrations of ACTH were preserved and comparable to after nebulised DSP administration. Studies evaluating the effects of inhaled fluticasone propionate on basal cortisol in horses have given contradictory results with some studies documenting HPA axis suppression [12,30,31], while others did not document significant decreases in basal cortisol concentrations [32].

Use of systemic formulations of drugs for nebulisation can lead to exposure to potentially irritant substances, toxic additives and inappropriate pH or osmolality ranges. Airway neutrophilia in response to irritants occurs rapidly in horses and BAL cytology is a sensitive indicator of diffuse airway inflammation. In one study a single instillation of 0.9% NaCl in the lungs resulted in a significant increase in the number and proportion of neutrophils in BAL fluid for at least 48 h [33]. In the present study nebulisation with the DSP formulation commercially available for systemic use for five consecutive days did not result in clinical signs of respiratory disease or airway inflammation as assessed by cytological examination of BAL fluid. Mean baseline percentage of neutrophils (14.7  $\pm$  5.5%) in the horses of this study was higher than the upper limit of the reference range [1] indicating some degree of underlying inflammation despite normal physical examination and absence of clinical signs even during exercise. The percentage of neutrophils in BAL fluid decreased significantly during the treatment period regardless of route of administration. This decrease might be the result of the anti-inflammatory properties of dexamethasone as both systemic and nebulised corticosteroids have been shown to decrease airway neutrophilia in horses with asthma [11,34]. Alternatively, the decrease in the percentage of neutrophils might just be the result of environmental changes as the horses were removed from pasture 24 h prior to the beginning of the study and stalled during the experimental period. Typically, however, stabling is associated with a significant increase rather than a decrease in the percentage of neutrophils in BAL fluid in both healthy horses and horses with heaves [35]. Although considerably safer than systemic corticosteroids, inhaled corticosteroids are not necessarily innocuous with adverse effects including decreased growth rate, decreased bone density and HPA suppression reported in people [36].

In conclusion, DSP administered via nebulisation had minimal systemic bioavailability and did not induce lower airway inflammation or HPA axis suppression in healthy horses. Additional studies will be needed to assess the clinical efficacy and safety of long-term nebulised DSP in horses with asthma before it is recommended for clinical use. Nebulisation of DSP to horses would represent off label use of the drug with rules and regulations varying from country to country.

## Author's declarations of interest

No competing interests have been declared.

## Ethical animal research

The study was approved by the Clinical Research Committee of the University of Georgia.

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## Authorship

A. Haspel, S. Giguère, K. Hart, L. Berghaus and J. Davis contributed to study design, data collection and manuscript preparation.

## Manufacturers' addresses

<sup>a</sup>Diagnosics Product Corporation, Los Angeles, California, USA.

<sup>b</sup>Henry Schein Animal Health, Dublin, Ohio, USA.

<sup>c</sup>Nortev, Galway, Ireland.

<sup>d</sup>Malvern Instrument Limited, Malvern, Worcestershire, UK.

<sup>e</sup>PK Solutions 2.0, Summit Research Services, Montrose, Colorado, USA.

<sup>f</sup>Henry Schein Animal Health, Dublin, Ohio, USA.

<sup>g</sup>Jorgenson laboratories, Loveland, Colorado, USA.

<sup>h</sup>Nexelom Bioscience, Lawrence, Massachusetts, USA.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Supplementary Item 1:** Measurement of dexamethasone concentration in plasma by ultra-performance liquid chromatography with tandem mass spectrometry (UPLC/MS-MS).