Pharmacokinetics and pharmacodynamics of dexamethasone after oral administration in apparently healthy horses

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Objective—To assess pharmacokinetic and pharmacodynamic properties of dexamethasone administered PO as a solution or powder, compared with properties of dexamethasone solution administered IV, in apparently healthy horses.

Animals—6 adult horses.

Procedures—Serum cortisol concentration for each horse was determined before each treatment (baseline values). Dexamethasone (0.05 mg/kg) was administered PO (in solution or powdered form) or IV (solution) to horses from which feed had or had not been withheld (unfed and fed horses, respectively). Each horse received all 6 treatments in random order at 2-week intervals; PO and IV administrations of dexamethasone were accompanied by IV or PO sham treatments, respectively. Plasma dexamethasone and serum cortisol concentrations were assessed at predetermined intervals.

Results—Maximum plasma dexamethasone concentration after PO administration of powdered dexamethasone in unfed horses was significantly higher than the maximum plasma concentration after PO administration of dexamethasone solution in unfed or fed horses. Mean bioavailability of dexamethasone ranged from 28% to 66% but was not significantly different among horses receiving either formulation PO in the unfed or fed state. After dexamethasone treatment PO or IV, serum cortisol concentrations were significantly less than baseline at 1 to 72 hours in unfed horses and at 2 to 48 hours in fed horses.

Conclusions and Clinical Relevance—PO or IV administration of dexamethasone resulted in suppression of cortisol secretion in unfed and fed adult horses; the magnitude of suppression did not differ among treatment groups, and serum cortisol concentrations returned to baseline after 48 to 72 hours. (*Am J Vet Res* 2010;71:831–839)

Therapeutic immune suppression via administration of steroidal anti-inflammatory medications is necessary for a variety of conditions in horses, including recurrent airway obstruction (ie, heaves), interstitial pneumonia, immune-mediated diseases, hypersensitivity reactions, and inflammatory airway disease. Treatment for these conditions is typically initiated with IV administration of glucocorticoids and subsequently modified to a lower dose of PO administered medication, which may be continued for a period of several days to weeks until it is gradually discontinued by use of tapering doses. Although preparations of glucocorticoids for PO administration are commercially produced and approved by the FDA for human or veterinary use, the use of such products may not be economically feasible or the products may not be readily available or efficacious. For example, the FDA-approved powdered formulation of dexamethasone[a] is difficult to ob-

Abbreviations

AUC	Area under the plasma concentration-versus-time curve
$AUC_{0-\infty}$	Area under the plasma concentration-versus-time curve from time 0 to infinity
C_{max}	Maximum plasma concentration of dexamethasone
IS	Internal standard
LC	Liquid chromatography
LC-MS	Liquid chromatography–mass spectrometry
MRT	Mean residence time
PPID	Pituitary pars intermedia dysfunction
$t_{½\lambda z}$	Terminal half-life
T_{max}	Time to maximal measured plasma dexamethasone concentration
Vd_{ss}	Volume of distribution at steady state

Received April 15, 2009.
Accepted May 15, 2009.
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Presented in abstract form at the American College of Veterinary Internal Medicine Conference, Seattle, June 2007.
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tain due to variable product availability; therefore, an approved human drug or a legally compounded formulation may be substituted. Other FDA-approved preparations available for PO administration in humans include prednisone, which is poorly absorbed in horses,[1] and prednisolone, the cost of which may be prohibitive for extended use. Because of these issues, clinicians in equine practice often administer the injectable formulation of dexamethasone solution (approved by the FDA for use IV or IM) PO to horses. This extralabel treatment is performed despite very limited investigation into its pharmacological effects.

Endogenous glucocorticoids are produced by the adrenal cortex and enter the blood in a circadian pattern.[2,3] Glucocorticoids exert their immunosuppressive and anti-inflammatory properties by binding to glucocorticoid receptors within the cytoplasm of targeted cells and altering gene expression or repression through genomic control of mRNA production.[2,4–7] The genomic effects of glucocorticoids generally require a period of hours to days to become detectable, and in recent years, nongenomic pathways have been recognized as having an important role in the more rapid onset of glucocorticoid effects. Glucocorticoids bind membrane receptors in nongenomic pathways that rapidly stimulate second messengers and electrolyte transfer, rather than alter mRNA production.[6,8]

Dexamethasone, a fluorinated analog of prednisolone, has a longer duration of activity and is approximately 30 times as potent as endogenous cortisol.[9] In horses, exogenous systemic glucocorticoid administration has been associated with adverse effects such as hypothalamic-pituitary-adrenal axis suppression, muscle wasting, hyperglycemia, polyuria, polydipsia, immunosuppression, and laminitis.[5,10–17] Adrenocortical dysfunction is assessed via the evaluation of endogenous cortisol response to ACTH administration, and studies[11,14,17] have been performed to investigate adrenocortical function in response to exogenous glucocorticoids in horses. Suppression of cortisol secretion, which indicates adrenal gland suppression, develops subsequent to administration of glucocorticoids via IV, IM, or inhalant methods.[11,12,14–17] Short-term parenteral administration of dexamethasone, prednisolone sodium succinate, or aerosolized beclomethasone dipropionate has not been associated with adrenocortical dysfunction as evaluated by response to ACTH stimulation testing.[11,12,14–17] However, a single dose of prednisolone acetate or triamcinolone acetonide has resulted in adrenocortical dysfunction for 14 to 21 days.[14,16,18,19]

To the authors' knowledge, the pharmacokinetics and pharmacodynamics of PO administered dexamethasone solution in horses have not been completely determined. Pharmacokinetic studies[1,11,14,15,20,21] have been reported for dexamethasone solution administered IM or IV and for dexamethasone powder, prednisone, or prednisolone tablets administered PO. Other studies[1,21–23] revealed that prednisone administered PO has limited efficacy in horses, but PO administration of the dexamethasone solution formulated for injection is effective against inflammatory conditions and provides an economical and convenient means of glucocorticoid delivery.[24]

Although there are few clinical studies[24,25] evaluating the pharmacological effects and efficacy of dexamethasone solution given PO to horses, this method of administration is becoming more common in equine practice. The standard for dose determination of glucocorticoids given PO is based on the bioavailabilities of dexamethasone powder and prednisolone, which are each approximately 61%.[1,20] The focus of the study reported here was to compare the pharmacokinetics of dexamethasone solution administered PO, dexamethasone powder administered PO, and dexamethasone solution administered IV in apparently healthy horses. In addition, pharmacodynamic effects were evaluated by determination of systemic cortisol responses to the different formulations and routes of administration of dexamethasone.

Materials and Methods

Animals—Five Quarter Horses and 1 Dutch Warmblood (4 mares and 2 geldings; age range, 13 to 27 years; weight, 385 to 630 kg) from the herd at a veterinary medical teaching hospital were included in the study. Horses did not have clinical signs consistent with PPID and were determined to be healthy on the basis of the results of physical examination, a CBC, and serum biochemical analysis. However, PPID was later diagnosed in one of the horses. All horses were accustomed to handling and venipuncture. Each horse received 3 independent treatments after feed had or had not been withheld (ie, in the unfed and fed states, respectively). The effects of study design and hospitalization on circadian rhythms of cortisol secretion were subsequently evaluated in 5 of the 6 horses included in the study, without administration of dexamethasone. One of the horses in the study was adopted and was eliminated from the study prior to analysis of circadian rhythm; however, all of the dexamethasone treatment experiments had been completed.

All horses were allowed free access to individual paddocks, except during the experimental periods when horses were housed in individual stalls. Each experimental period began 18 hours prior to drug administration and ended when sample collection was completed. All horses had access to fresh water at all times and were fed their typical complete pelleted diet in 2 equal feedings with grass hay available ad libitum, except when feed was withheld for an 11-hour period at the start of experiments that involved horses in the unfed state. For each of those experiments, withholding of feed began 8 hours prior to drug administration and ended 3 hours after drug administration. The study was approved by the Institutional Animal Care and Use Committee at Kansas State University.

Drug administration—In each experiment, horses in the unfed or fed state received 1 of 3 treatments: a commercially available injectable formulation of dexamethasone solution[b] administered at a dose of 0.05 mg/kg, PO; dexamethasone powder[c] administered at a dose of 0.05 mg/kg, PO; and dexamethasone solution[b] administered at dose of 0.05 mg/kg, IV. Treatments were administered in random order as part of a randomized crossover block design with a minimum washout period

of 2 weeks between treatments.[20] To minimize changes attributable to normal circadian rhythm, all experiments started at 8:00 AM. Intravenous administration of dexamethasone solution was performed by 1 investigator (JAG) via venipuncture of the left jugular vein. Administration of dexamethasone solution or powder PO was performed by the same investigator (JAG) by use of a syringe designed for PO delivery of medications. The solution was administered PO without mixing with molasses or water. The FDA-approved commercially formulated dexamethasone powder[a] was not available because of a lack of manufacturer production; a compounded formulation[c] (10 mg/teaspoon; 1 teaspoon = 2.56 g) derived from bulk pharmaceutical ingredients was obtained from a reputable veterinary compounding pharmacy and was mixed in molasses prior to PO administration. The concentration of dexamethasone was determined by licensed pharmacists at the pharmacy and not further validated by the authors. The authors understand the FDA regulations associated with the use of bulk pharmaceutical compounds in the production of veterinary pharmaceuticals and recognize the importance of using FDA-approved dexamethasone products that may be easily administered PO. However, it is the authors' experience that when the approved dexamethasone product cannot be obtained, compounded formulations are prescribed for PO administration by owners instead.

When dexamethasone was administered PO, a sham treatment of saline (0.9% NaCl) solution (volume equivalent to that of the dexamethasone solution [2 mg/mL] administered IV at a dose of 0.05 mg/kg) was also administered IV. When dexamethasone was administered IV, a sham treatment of liquid molasses (volume equivalent to that of the powder mixed with molasses administered PO at a dose of 0.05 mg/kg) was given PO.

Sample collection and determination of drug concentrations—Blood samples (10 mL each) were obtained from each horse by use of a 14-gauge, 5.25-inch catheter inserted into the right jugular vein. Prior to catheter placement, an area over the vein was clipped, aseptically prepared with 4% chlorhexidine gluconate solution, and rinsed with 70% isopropyl alcohol, and the skin was infiltrated with 2% lidocaine HCl solution[d] ID. For each experiment, an initial blood sample was obtained from each horse while it was in its typical environment outside the hospital 24 hours before drug administration. Intravenous catheters were placed prior to drug administration and removed following the last sample collection for each experiment. Serial blood samples were then collected immediately prior to drug administration (designated as time 0) and at 15, 30, and 45 minutes and 1, 2, 4, 8, 12, 24, 36, 48, and 72 hours after drug administration. Blood samples were immediately transferred to sodium-heparin–coated tubes and serum separator tubes. After centrifugation for 5 minutes, plasma and serum samples were frozen and stored at –70°C until analyzed.

Plasma dexamethasone concentrations were determined with LC-MS. The mobile phase consisted of 70% methanol in 0.1% acetic acid solution with a flow rate of 0.3 mL/min. Separation was achieved on a 150 × 2.1-mm, 3-µM C8 column.[e] The IS solution (100 µL of a solution containing 100 ng of desoximetasone/mL) was added to 1-mL plasma samples, which were then subjected to solid-phase extraction. The solid-phase extraction cartridges[f] were conditioned with 1 mL of methanol and 1 mL of deionized water, and the plasma samples with IS solution were loaded. The cartridges were washed with a 5% methanol solution, and samples were eluted with 1 mL of methanol. Eluates were evaporated for 30 minutes at 40°C under a stream of air to a dry state and then reconstituted with 0.2 mL of a 50% methanol solution; 0.05 mL of each sample was injected into the analyzer. The spectrometric transitions quantified for dexamethasone and the IS solution (mass-to-charge ratios) were 393 to 373 and 377 to 357, respectively, as previously described.[26] The standard curves were linear from 1 to 50 ng/mL, with measured concentrations within 15% of actual concentrations and a correlation coefficient (r) ≥ 0.99; therefore, the lower limit of quantification was 1 ng/mL. Accuracy and coefficient of variation were determined for 5 replicates each at 1, 10, and 50 ng/mL. Mean ± SD accuracy was within 9 ± 6% of the actual value, whereas the coefficient of variation was 7 ± 2%. Serum cortisol concentrations were analyzed by use of a chemiluminescent enzyme immunoassay,[g] as previously described,[11,16] with a quantification range of 5.5 to 1,380 nmol/L.

Evaluation of circadian rhythms and effect of experimental design—To evaluate the effects of hospitalization, administration methods, and sample collection on suppression of cortisol release, control experiments without dexamethasone administration were performed after a washout period of 2 months. This aspect of the study was performed after the completion of sample collections for the dexamethasone treatment experiments. One horse was adopted in the interim and was excluded from this part of the study. All other horses used in the dexamethasone treatment experiments were included; therefore, 5 of the 6 horses that met the study criteria completed all portions of the study. Intravenous catheters were aseptically placed as previously described. A sham treatment of saline solution (volume equivalent to that of the dexamethasone solution [2 mg/mL] administered IV at a dose of 0.05 mg/kg) was given IV by 1 investigator (JAG), and a sham treatment of liquid molasses (volume equivalent to that of the powder mixed with molasses administered PO at a dose of 0.05 mg/kg) was given PO (JAG) to each horse to mimic administration of IV dexamethasone solution and PO dexamethasone solution or powder immediately prior to time 0. Blood samples were collected every 2 hours for 48 hours, and samples were handled and prepared as previously described for analysis of cortisol concentrations.

Pharmacokinetic analysis—Plasma dexamethasone concentration versus time data were analyzed for each horse after each treatment by use of computer software[h] to estimate parameters. The plasma concentrations extrapolated by log-linear regression to time 0, $AUC_{0-\infty}$, clearance, $t_{½\lambda z}$, MRT, and Vd_{ss} for dexamethasone administered IV were calculated via a noncompartmental method. The bioavailability (fraction of

the dose absorbed [F]) for dexamethasone solution or powder administered PO was calculated by use of an equation as follows: F (%) = 100 × (AUC_{PO}/AUC_{IV}), where AUC_{PO} is the AUC following PO administration and AUC_{IV} is the AUC following IV administration. The T_{max} and C_{max} of dexamethasone following PO administration were determined from actual measured data points. The mean absorption time for dexamethasone administered PO was determined by subtracting the MRT for dexamethasone given IV from the MRT for the formulation that was given PO.

For dexamethasone administered PO, a noncompartmental method was also used to determine the $AUC_{0-\infty}$, volume of distribution (area method) per fraction of the dose absorbed (Vz/F), and clearance per fraction of the dose absorbed (Cl/F). The $AUC_{0-\infty}$ was determined via the linear trapezoidal rule, regardless of the route of administration. Clearance was calculated as dose/AUC. The Vd_{ss} was calculated as MRT × clearance. The $t_{½\lambda z}$ was calculated by use of an equation as follows: $t_{½\lambda z}$ = 0.693/λz, where λz is the first-order rate constant.

Pharmacodynamic analysis—Suppression of cortisol release from the adrenal cortex was analyzed via evaluation of actual measured data points for serum cortisol concentrations in response to administration of dexamethasone.

Statistical analysis—Statistical analysis was performed by the use of computerized statistical programs.[h,i] Comparisons of pharmacokinetic variables were performed for all treatment groups (dexamethasone solution administered PO, dexamethasone powder administered PO, and dexamethasone solution administered IV in unfed and fed horses) via nonparametric Kruskall-Wallis 1-way ANOVA. When differences were significant ($P < 0.05$) for these variables, pairwise multiple comparison procedures were performed via a Bonferroni-Dunn test. To evaluate suppression of cortisol secretion, comparisons of pharmacodynamic variables were performed for all treatment groups via the mixed procedure for repeated measures. When significant ($P < 0.05$) differences were determined for these responses, pairwise multiple comparison procedures were performed via a least square means algorithm. Mean serum cortisol concentrations at each time point for unfed and fed horses that received each treatment were compared via a Student *t* test. The circadian rhythm of cortisol secretion was evaluated via a 1-way ANOVA with a Tukey test for multiple comparisons.

Results

Dexamethasone was tolerated well by all horses after PO or IV administration. No adverse effects were detected.

Mean plasma dexamethasone concentrations at predetermined time points following IV or PO administration of the different dexamethasone formulations were plotted for unfed and fed horses (**Figure 1**). Plasma dexamethasone concentrations were less than the limit of quantification (ie, 1 ng/mL) 8 hours after administration in unfed horses treated with dexamethasone solution PO. Plasma dexamethasone concentrations were < 1 ng/mL 12 hours following dexamethasone administration for all other treatment groups.

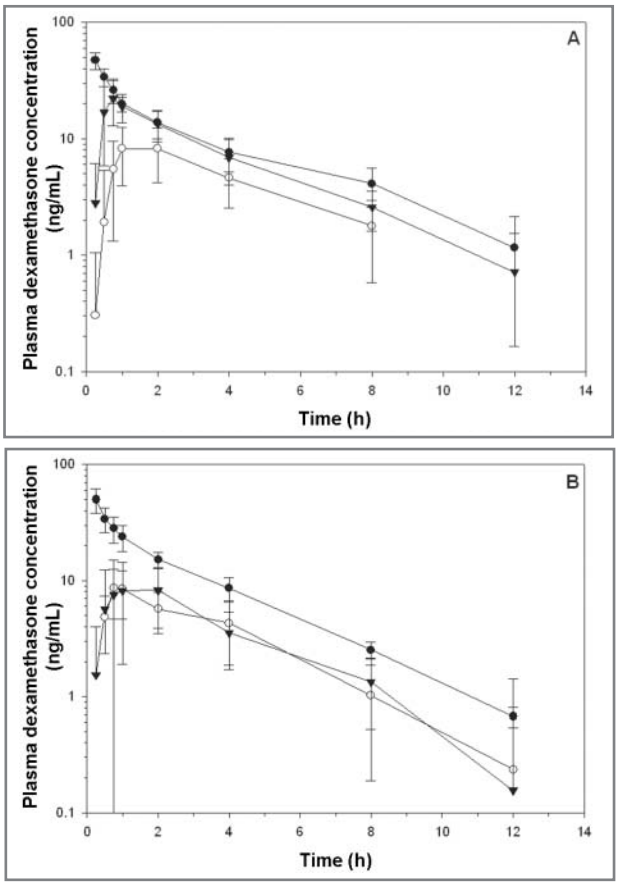

Figure 1—Mean ± SD plasma dexamethasone concentrations obtained immediately prior to administration (designated as time 0) of dexamethasone (0.05 mg/kg) and at predetermined intervals thereafter in 6 horses. Dexamethasone solution (white circles) or dexamethasone powder (black triangles) was administered PO or dexamethasone solution was administered IV (black circles) to horses from which feed had (A) or had not (B) been withheld (unfed and fed states, respectively) as part of a randomized, crossover block design study. For unfed horses, feed was withheld from 8 hours prior to treatment until 3 hours after treatment; for fed horses, pelleted feed was provided twice daily with hay ad libitum. When dexamethasone was administered PO, a volume of saline (0.9% NaCl) solution equivalent to that of the calculated dose of dexamethasone solution was injected IV. When dexamethasone was administered IV, a volume of liquid molasses equivalent to the volume of the powder mixed with molasses was given PO.

Noncompartmental pharmacokinetic parameters were determined for all dexamethasone treatment groups (**Tables 1 and 2**). The geometric mean of plasma dexamethasone concentration following IV administration was 64.52 and 71.78 ng/mL for unfed and fed horses, respectively. Mean C_{max} values for the unfed and fed horses that received dexamethasone solution PO (8.57 and 8.90 ng/mL, respectively) were significantly (P = 0.006) different from the mean value for unfed horses (22.08 ng/mL) that received dexamethasone powder PO. However, there was no significant difference (P = 0.057) in mean T_{max} (range, 0.79 to 1.41 hours) between unfed and fed horses receiving either formulation PO. Following IV administration, a significant (P = 0.026) difference in mean $t_{½\lambda z}$ was detected between the unfed (3.40 hours) and fed (2.52 hours) horses; however,

Table 1—Noncompartmental pharmacokinetic variables (geometric mean, median, minimum [Min], and maximum [Max]) after IV or PO administration of dexamethasone (0.05 mg/kg) as a powder (PO) or a solution (PO or IV) in 6 horses from which feed was withheld from 8 hours prior to treatment until 3 hours after treatment (unfed state).

	Dexamethasone formulation (route of administration)											
	Solution (IV)				Solution (PO)				Powder (PO)			
Variable	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max
AUC_{Extrap} (%)	8.92	8.01	4.71	27.87	15.03	13.51	8.11	25.75	8.22	9.64	4.54	12.99
$AUC_{0-\infty}$ (h•ng/mL)	118.18	116.79	90.52	156.93	52.23	47.25	38.11	75.33	75.86	72.80	52.88	129.61
$AUMC_{0-\infty}$ (h•h•ng/mL)	463.11	484.73	254.64	721.69	277.70	273.92	159.39	447.32	292.53	266.24	182.49	489.26
C_0 (ng/mL)	64.52	62.10	46.70	87.97	—	—	—	—	—	—	—	—
Cl (mL/min/kg)	7.05	7.18	5.31	9.21	—	—	—	—	—	—	—	—
Cl/F (mL/min/kg)	—	—	—	—	15.95	17.64	11.06	21.87	10.99	11.48	6.43	15.76
C_{max} (ng/mL)	—	—	—	—	8.57	10.54	2.46	13.48	22.08	21.07	16.37	40.04
$t_{½\lambda z}$ (h)	3.40	3.54	2.48	4.31	3.66	3.72	2.35	8.05	2.57	2.51	2.32	3.13
λ_z (1/h)	0.20	0.20	0.16	0.28	0.19	0.19	0.09	0.29	0.27	0.28	0.22	0.30
$MRT_{0-\infty}$ (h)	3.92	3.96	2.81	5.71	5.32	5.14	4.14	8.05	3.86	3.72	3.45	4.86
T_{max} (h)	—	—	—	—	1.41	1.50	1.00	2.00	0.79	0.75	0.75	1.00
Vd_{ss} (L/kg)	1.66	1.59	1.29	2.26	—	—	—	—	—	—	—	—
V_z (L/kg)	2.08	2.12	1.62	2.46	—	—	—	—	—	—	—	—
V_z/F (L/kg)	—	—	—	—	5.06	4.45	2.72	12.29	2.45	2.50	1.46	3.17
F (%)	—	—	—	—	42	44	23	60	66	76	32	95
MAT (h)	—	—	—	—	—	0.33	–0.57	3.75	—	0.35	–2.05	0.84

When dexamethasone was administered PO, a volume of saline (0.9% NaCl) solution equivalent to that of the calculated dose of dexamethasone solution was injected IV. When dexamethasone was administered IV, a volume of liquid molasses equivalent to the volume of the powder mixed with molasses was given PO.

AUC_{Extrap} = Area under the curve from time 0 to infinity extrapolated from the last time point. $AUMC_{0-\infty}$ = Area under the first moment curve from 0 to infinity. C_0 = Plasma concentration of dexamethasone extrapolated to time 0. Cl = Plasma clearance of dexamethasone. F = Bioavailability (fraction of dose absorbed). λ_z = First-order rate constant. MAT = Mean absorption time. $MRT_{0-\infty}$ = Mean residence time from time 0 to infinity. V_z = Apparent volume of distribution of the area under the curve during the elimination phase following IV administration of dexamethasone. V_z/F = Apparent volume of distribution of the area under the curve during the elimination phase following PO administration of dexamethasone. — = Not applicable.

Table 2—Noncompartmental pharmacokinetic variables (geometric mean, median, minimum, and maximum) after administration of dexamethasone (0.05 mg/kg) as a powder (PO) or a solution (PO or IV) in the 6 horses in Table 1 after feed was not withheld at the start of the experiments (fed state).

	Dexamethasone formulation (route of administration)											
	Solution (IV)				Solution (PO)				Powder (PO)			
Variable	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max
AUC_{Extrap} (%)	5.49	5.39	4.15	8.42	12.50	12.93	5.44	21.39	16.49	16.87	8.75	31.05
$AUC_{0-\infty}$ (h•ng/mL)	115.24	122.94	92.31	134.83	39.70	47.53	17.21	63.99	37.99	42.95	18.17	62.12
$AUMC_{0-\infty}$ (h•h•ng/mL)	333.64	363.06	245.32	423.58	160.24	186.50	45.11	366.68	171.30	176.30	89.71	291.29
C_0 (ng/mL)	71.78	74.01	48.38	99.95	—	—	—	—	—	—	—	—
Cl (mL/min/kg)	7.23	6.78	6.18	9.03	—	—	—	—	—	—	—	—
Cl/F (mL/min/kg)	—	—	—	—	20.99	17.57	13.02	48.44	21.93	19.87	13.42	45.86
C_{max} (ng/mL)	—	—	—	—	8.90	8.70	4.41	14.73	8.50	12.74	1.46	18.42
$t_{½\lambda z}$ (h)	2.52	2.56	2.12	2.87	2.29	2.25	1.52	5.02	2.55	2.79	1.04	4.21
λ_z (1/h)	0.28	0.27	0.24	0.33	0.30	0.31	0.14	0.46	0.27	0.26	0.16	0.67
$MRT_{0-\infty}$ (h)	2.90	2.84	2.47	3.46	4.04	3.90	2.62	7.37	4.51	4.15	2.47	9.88
T_{max} (h)	—	—	—	—	1.02	1.00	0.75	2.00	1.20	1.50	0.50	2.00
Vd_{ss} (L/kg)	1.26	1.35	0.91	1.44	—	—	—	—	—	—	—	—
V_z (L/kg)	1.58	1.61	1.23	1.88	—	—	—	—	—	—	—	—
V_z/F (L/kg)	—	—	—	—	4.17	4.70	2.03	7.27	4.83	3.54	2.07	16.69
F (%)	—	—	—	—	31	33	15	67	28	37	10	56
MAT (h)	—	—	—	—	—	0.84	0.05	4.35	—	1.15	0.20	7.41

See Table 1 for key.

there was no significant ($P = 0.211$) difference in mean $t_{½\lambda z}$ between unfed and fed horses that received dexamethasone solution (3.66 and 2.29 hours, respectively) or powder (2.57 and 2.55 hours, respectively) PO. Following IV administration of dexamethasone, mean clearance was 7.05 and 7.23 mL/min/kg, mean Vd_{ss} was 1.66 and 1.26 L/kg, and mean $AUC_{0-\infty}$ was 118.18 and 115.24 h•ng/mL in unfed and fed horses, respectively. The mean $AUC_{0-\infty}$ for unfed and fed horses administered dexamethasone solution PO was 52.23 and 39.70 h•ng/mL, respectively. There was a significant ($P = 0.033$) difference in mean $AUC_{0-\infty}$ between unfed (75.86 h•ng/mL) and fed (37.99 h•ng/mL) horses that were administered dexamethasone powder PO. The

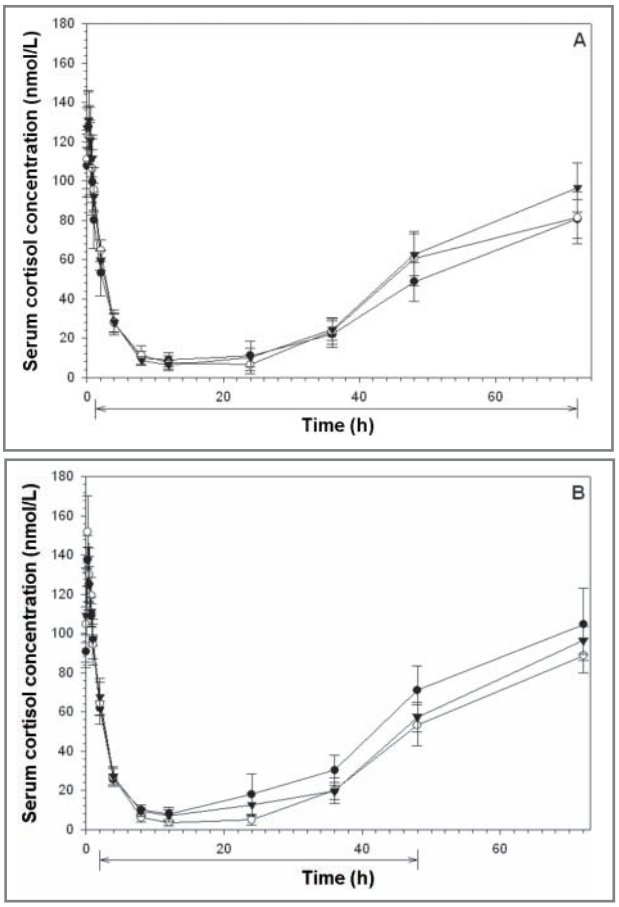

Figure 2—Mean ± SD serum cortisol concentrations in the 6 horses in Figure 1. Horses in an unfed (A) or fed (B) state were administered various formulations of dexamethasone at a dose of 0.05 mg/kg (solution [PO], powder [PO], or solution [IV]) as part of a randomized, crossover block design study. Time points spanned by the double-headed arrow indicate intervals when concentrations were significantly ($P < 0.05$) different from baseline, indicating adrenal gland suppression. *See* Figure 1 for key.

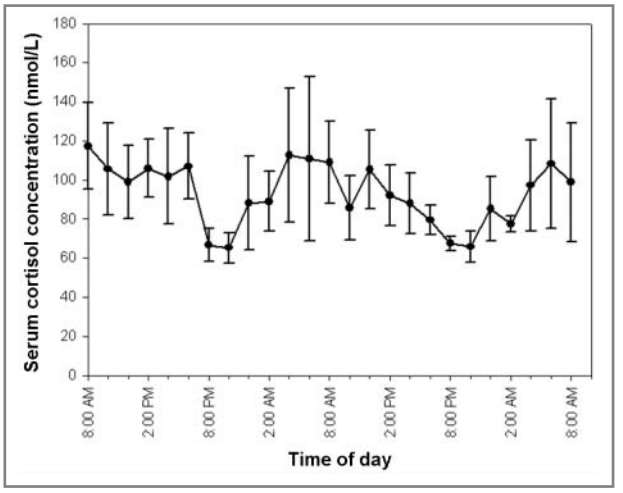

Figure 3—Mean ± SD serum cortisol concentrations assessed 2 months after completion of the dexamethasone treatment experiments in 5 of the 6 study horses to evaluate diurnal circadian variation of cortisol secretion during hospitalization, sham treatment, and sample collection without dexamethasone administration. A sham treatment of saline solution was given IV and a sham treatment of liquid molasses was given PO to each horse to mimic dexamethasone solution administered IV and dexamethasone solution or powder administered PO immediately prior to start of the 48-hour assessment period. Blood samples were collected every 2 hours for 48 hours.

mean bioavailability (fraction of the dose absorbed) for the PO administered dexamethasone solution was 42% and 31% for unfed and fed horses, respectively; bioavailability for the PO administered powder was 66% in unfed horses and 28% in fed horses ($P = 0.050$).

Endogenous serum cortisol concentrations at the various time points following dexamethasone administration in all treatment groups were plotted (**Figure 2**). Baseline serum cortisol concentrations were similar among all treatment groups; therefore, any residual effects of dexamethasone on these results were minimal. There was no significant difference in suppression of cortisol concentrations among treatment groups. However, there was a significant decrease from mean baseline in serum cortisol concentration for all unfed horses (regardless of treatment) from 1 hour after dexamethasone administration until 72 hours after administration. A significant decrease from mean baseline in serum cortisol concentrations was also observed for all fed horses (regardless of treatment), beginning 2 hours after dexamethasone administration and continuing until 48 hours after administration.

Hospitalization, administration of medications, or sample collection did not have an effect on the circadian rhythm of cortisol secretion of the horses in the study. Evaluation of endogenous serum cortisol concentrations for patterns indicative of circadian rhythms revealed a diurnal variation. Mean cortisol concentrations at 6:00 AM and 8:00 AM were significantly higher than those at 8:00 PM and 10:00 PM. Similarly, serum cortisol concentrations at 12:00 PM were significantly higher than at 10:00 PM (**Figure 3**).

Discussion

The study reported here was designed to determine and compare the pharmacokinetics and bioavailability of dexamethasone solution administered PO, dexamethasone powder administered PO, and dexamethasone solution administered IV in apparently healthy horses. Furthermore, pharmacodynamic properties were evaluated for each treatment by assessing adrenal gland suppression, which was determined on the basis of changes in endogenous serum cortisol concentrations. Additionally, the absorption of dexamethasone administered PO was evaluated by administering each treatment to each horse after feed was withheld or not withheld (ie, in the unfed or fed state, respectively), and the effect of these variations on the suppression of serum cortisol secretion was examined.

Because of the apparent health of the horses, lack of detectable clinical signs associated with PPID, and the inherent difficulty in diagnosing PPID antemortem, adrenal gland function was not assessed prior to commencing the study. One of the horses in the study had a weaker cortisol suppression response to dexamethasone administration than the other horses and had consistently higher serum cortisol concentrations than the remainder of treated horses, regardless of the formulation of dexamethasone or route of administration.

This 27-year-old horse was euthanatized approximately 6 months later for reasons unrelated to the study, and PPID was diagnosed. Because cortisol response after dexamethasone administration was suppressed in the aforementioned horse (although not to the extent of the other horses in the treatment group), the plasma dexamethasone and serum cortisol concentrations were still factored into the pharmacokinetic and pharmacodynamic analyses. However, the authors realize that inclusion of the data from the horse with PPID is a limitation of the study.

The circadian rhythm of circulating glucocorticoid concentrations has been reported[3,9,27,28] in humans, rhesus monkeys, rats, dogs, mice, channel catfish, and swine. Investigators in some studies successfully identified circadian rhythms in horses, although others did not.[3,28–31] In studies[3,31] that did not reveal a consistent pattern, it is possible that the sampling method and associated stress on untrained horses contributed to the apparent lack of a circadian rhythm. In the studies[3,28–30] in which a circadian rhythm was identified, peak concentrations were detected between 6:00 AM and 9:00 AM and trough concentrations were detected between 4:00 PM and 11:00 PM. The horses in the study reported here maintained a circadian rhythm of cortisol secretion during hospitalization. However, it is important to recognize that, as part of a teaching herd, they were accustomed to handling, venipuncture, and hospitalization. These results suggest that there is a circadian rhythm of cortisol secretion in horses with a peak concentration in the morning and a trough concentration in the evening, but it may easily be disrupted when a horse is placed in stressful situations. The investigation described in this report appeared to have minimal effects on the natural circadian rhythm of cortisol secretion in this group of horses.

Until recently, pharmacokinetic and pharmacodynamic analysis of dexamethasone in horses was considered to be incomplete because of the lack of sensitivity of previous analytic methods. However, in 2005, Soma et al[15] used LC-MS with a sensitivity of 100 pg/mL to evaluate the pharmacokinetic and pharmacodynamic effects of dexamethasone administered IV in horses. Investigators in previous studies[14,20] determined the pharmacokinetics of dexamethasone by use of less sensitive high-pressure LC or radioimmunoassays; the study[15] by Soma et al was integral in providing complete pharmacokinetic analysis following IV administration of dexamethasone in horses. Compared with the results of other studies[14,15,20] that were performed to evaluate the pharmacokinetic properties of dexamethasone injected IV, the pharmacokinetic results from the present study (ie, plasma concentration at time 0, clearance, Vd_{ss}, MRT, and AUC) were similar to those found by Soma et al[15] by use of LC-MS, as well as those revealed in a different study.[20] Therefore, the use of LC-MS with a minimum limit of quantification of 1 ng/mL allowed an accurate characterization of the pharmacokinetic nature of dexamethasone in horses.

To the investigators' knowledge, the study reported here is the first to evaluate the pharmacokinetics of dexamethasone solution administered PO in horses. Cunningham et al[20] performed a pharmacokinetic study of dexamethasone powder[a] administered PO in horses, and pharmacokinetic analysis of prednisolone administered PO was reported[1] several years later. Those 2 studies,[1,20] which revealed bioavailabilities of approximately 61% for the commercially available dexamethasone powder[a] and for prednisolone, have served as benchmarks for the determination of doses of PO administered glucocorticoids in horses. The mean ± SD T_{max} and the elimination half-life reported by Cunningham et al[20] (1.3 ± 0.5 hours and 4.36 ± 1.34 hours) were similar to those in the present study for all horses receiving PO treatment with dexamethasone (mean T_{max} range, 0.79 to 1.41 hours and mean $t_{1/2\lambda z}$ range, 2.29 to 3.66 hours). For all horses administered dexamethasone PO in the present study, the mean C_{max} ranged from 8.50 to 22.08 ng/mL, compared with 4.9 ng/mL for the dexamethasone powder[a] reported by Cunningham et al.[20] The 2 dexamethasone formulations administered PO in the study reported here resulted in greater means for $AUC_{0-\infty}$ (37.99 to 75.86 h•ng/mL), compared with the results of Cunningham et al[20] (29.09 ± 8.69 h•ng/mL). Mean bioavailability of dexamethasone powder after PO administration in the present study and in the Cunningham et al study[20] was extremely variable, ranging from 28% to 66% and 31% to 88%, respectively. Mean bioavailability of dexamethasone solution after PO administration in the present study was 42% in unfed horses and 31% in fed horses. Although no significant difference was detected in bioavailability of dexamethasone regardless of the formulation, route of administration, or status of the horses (unfed vs fed), the compounded powdered formulation resulted in significantly higher maximum plasma concentrations and had a higher bioavailability (66%) after administration to unfed horses.

Although the concentration of dexamethasone was determined by licensed pharmacists at the compounding pharmacy,[c] the actual concentration of dexamethasone in the compounded powder was not confirmed by the authors, and pharmacokinetic results for the powdered formulation should be interpreted cautiously because compounded formulations may not be as accurate as commercially available formulations.[32] Medications manufactured without FDA approval may result in the production of a product with varying concentration, ranging from no active ingredient to higher concentrations than described on the label.[33] However, because compounded formulations are administered to horses, the authors thought it was important to include this type of product in the study, despite its potential limitations. To minimize the effects of these potential limitations in future studies, an unbiased laboratory may be used to validate the concentrations of active ingredient for compounded medications.[33] The lack of significant differences in the bioavailability of the various formulations for PO administration may have been attributable to extreme variability in absorption of dexamethasone administered PO and the limited number of horses included in the study.

In the study reported here, as well as in other studies,[14,15] IV injection of dexamethasone resulted in significant adrenal gland suppression as determined by a decrease in serum cortisol concentrations from baseline, beginning 1 to 2 hours after administration. This

suppression was preceded by a slight increase in serum cortisol concentration at the first measured time point (15 minutes) after dexamethasone administration.[14,17] It is possible that this could be associated with the administration of the medication; alternatively, it has been proposed that this may be associated with normal intracircadian fluctuations with intermittent peaks and troughs of circulating cortisol concentrations.[3,15,30] Horses in the present study were accustomed to handling and venipuncture, and serial blood samples were obtained by the use of venous catheters. Investigators attempted to minimize excitement of the horses during administration of dexamethasone and during sample collection. Suppression of cortisol secretion in the present study was similar to that identified in another study[15] after IV administration of dexamethasone. For all unfed horses in the study reported here, significant decreases in mean circulating cortisol concentrations were detected beginning 1 hour after dexamethasone administration and continuing until 72 hours after administration. Regardless of the formulation administered, mean serum cortisol concentrations in fed horses were significantly suppressed from 2 to 48 hours after dexamethasone administration. Despite the fact that plasma dexamethasone concentrations were not detected in any unfed or fed horses > 12 hours after administration, cortisol secretion remained suppressed for 48 to 72 hours. These findings, together with the large Vd_{ss}, support the idea that intracellular dexamethasone concentration is more important than plasma concentration.[14,15] In addition, changes in transcription and translation may lag behind changes in circulating drug concentrations.

Because of the greater sensitivity of the analytic methods used by Soma et al,[15] those investigators identified a previously unreported third pharmacokinetic compartment for the distribution of dexamethasone after IV administration in horses. The identification of a biphasic (ie, 2-compartment) plasma profile for dexamethasone administered IV in the study reported here may have been caused by quantification limitations of the LC-MS used to determine drug concentrations. We speculated that the third compartment (which would be confirmed by detecting a triphasic plasma profile) was present, and distribution of the dexamethasone to this compartment may have contributed to the delayed recovery of serum cortisol concentrations.[15] The delayed recovery of serum cortisol concentrations could also be a result of genomic effects of drug administration and subsequent gene expression or gene repression.

Pharmacodynamic studies[12,13,17] have been performed in which investigators monitored the effects of parenterally administered glucocorticoids on pulmonary function in horses with recurrent airway obstruction and also determined adrenal gland suppression through the measurement of serum cortisol concentrations. In those studies, suppression of cortisol secretion was associated with clinical improvement. The results of other studies[24,25] indicated that circulating cortisol concentrations are positively associated with measured pharmacodynamic variables following PO administration of dexamethasone solution. However, it is important to be aware that the association between cortisol suppression and improved lung function does not necessarily indicate cause and effect or suggest that these values serve as specific markers of immune effects. The fact that these changes were detected in parallel suggests that cortisol suppression may serve as a surrogate marker for these effects, but further studies are needed.

These experiments effectively determined the pharmacokinetics of dexamethasone administered PO as a solution or powder and dexamethasone solution administered IV in horses from which food had or had not been withheld for an 11-hour period that began 8 hours prior to drug administration. Although dexamethasone powder administered PO in unfed horses resulted in increased plasma concentrations and bioavailability, compared with the results for dexamethasone solution PO, greater variability was detected among samples from horses given the powder and the difference in bioavailability was not significant. In addition, suppression of cortisol secretion subsequent to dexamethasone administration was similar among all treatment groups, indicating similar pharmacodynamic responses regardless of the plasma concentrations of dexamethasone detected.

It has been suggested that the duration of therapeutic effects of synthetic and natural glucocorticoids may be approximated via evaluation of the hypothalamic pituitary-adrenal axis suppressive activity and that the degree of adrenal gland suppression corresponds with the anti-inflammatory potency and metabolic half-life of the drug.[11,14,34] Based on analysis of the results of the present study, it is possible that lower doses or less frequent administration of dexamethasone could have similar pharmacodynamic effects in horses. Similarly, the duration of cortisol suppression detected in these experiments may indicate that administration of dexamethasone every 48 hours could be as effective as treatment every 24 hours, which may potentially minimize the adverse effects associated with glucocorticoid administration. However, these hypotheses were not tested. Therefore, future studies should focus on pharmacodynamic modeling of this drug at lower and less frequent doses. The pharmacokinetics and pharmacodynamics described for dexamethasone in this study, in combination with results of similar studies, will be useful in predicting pharmacodynamic effects and developing anti-inflammatory models for determining improved dose regimens.

a. Azium powder, Schering-Plough Animal Health Corp, Summit, NJ.
b. Dexamethasone injection, 2 mg/mL, Phoenix Pharmaceutical Inc, St Joseph, Mo.
c. Wickliffe Veterinary Pharmacy Inc, Lexington, Ky.
d. Hospira Inc, Lake Forest, Ill.
e. Supelco Discovery, Sigma Aldrich, St Louis, Mo.
f. Oasis HLB, Waters Corp, Milford, Mass.
g. Immulite, Diagnostic Products Corp, Los Angeles, Calif.
h. SigmaStat, version 3.1, Systat Software Inc, Point Richmond, Calif.
i. SAS, version 9.1, SAS Institute Inc, Cary, NC.

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