

Pharmacokinetics of intra-articular, intravenous, and intramuscular administration of triamcinolone acetonide and its effect on endogenous plasma hydrocortisone and cortisone concentrations in horses

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Objective—To compare pharmacokinetics of triamcinolone acetonide (TA) following IV, intra-articular (IA), and IM administration and determine its effect on plasma concentrations of hydrocortisone and cortisone.

Animals—6 Thoroughbreds.

Procedures—TA (0.04 mg/kg) was administered IV, IM, or IA, and plasma TA, hydrocortisone, and cortisone concentrations were determined.

Results—IV administration of TA was fitted to a 2-compartment model. Median distribution half-life was 0.50 hours (range, 0.24 to 0.67 hours); elimination half-life was 6.1 hours (range, 5.0 to 6.4 hours). Transfer half-life of TA from joint to plasma was 5.2 hours (range, 0.49 to 7.3 hours); elimination half-life was 23.8 hours (range, 18.9 to 32.2 hours). Maximum plasma concentration following IA administration was 2.0 ng/mL (range, 0.94 to 2.5 ng/mL), and was attained at 10 hours (range, 8 to 12 hours). Maximum plasma concentration following IM administration was 0.34 ng/mL (range, 0.20 to 0.48 ng/mL) and was attained at 13.0 hours (range, 12 to 16 hours); concentration was still quantifiable at 360 hours. Hydrocortisone plasma concentrations were significantly different from baseline within 0.75, 2, and 1 hours after IV, IA, and IM administration, respectively, and remained significantly different from baseline at 96 and 264 hours for IV and IA administration. Following IM administration of TA, plasma concentrations of hydrocortisone did not recover to baseline concentrations by 360 hours.

Conclusions and Clinical Relevance—Pharmacokinetics of TA and related changes in hydrocortisone were described following IV, IA, and IM administration. A single administration of TA has profound effects on secretion of endogenous hydrocortisone. (*Am J Vet Res* 2011;72:1234–1242)

Triamcinolone acetonide is an intermediate-acting corticosteroid and is 5 times as potent as hydrocortisone.¹ Triamcinolone acetonide is not a prodrug for triamcinolone but has distinctly different corticosteroid pharmacodynamic properties.^{2,3} The acetate and acetonide esters alter water and lipid solubility, thereby delaying absorption and prolonging the duration of action.⁴ Acetonide esters are the less soluble corticosteroid and are considered to have intermediate to long

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ABBREVIATIONS

AUC	Area under the time-concentration curve
IA	Intra-articular
T _{1/2}	Half-life
TA	Triamcinolone acetonide
V _c	Volume of the central compartment
V _{d_{ss}}	Volume of distribution at steady state

duration of action following IA or IM administration.^{5,6} Thus, TA is one of the preferred drugs for IA administration.⁷ In equine medical practice, corticosteroid treatment via IA administration is used in the management of exercise-associated articular osteoarthritis and related conditions; some have suggested that TA may be more beneficial and less detrimental to joints than is methylprednisolone.^{8,9}

Corticosteroids have appreciable immunosuppressive, anti-inflammatory, and lympholytic effects, including marked suppression of endogenous hydrocortisone. These effects are not minimized by IA or IM administration. Secondary hyperadrenocorticism, adrenal crisis, and anaphylaxis have been reported in

humans following IA, topical, or local administration of glucocorticoids.¹⁰⁻¹⁴ Decreased plasma concentrations of endogenous glucocorticoids following IA administration of methylprednisolone have been detected for up to 1 week in humans,¹⁵ 3 to 10 days in horses,^{16,17} and 12 weeks in cows.¹⁸ In horses, endogenous hydrocortisone does not reach pretreatment concentrations until 14 days after IM administration of TA.^{19,20} Topical and ophthalmic applications of TA or dexamethasone suppress endogenous cortisol concentrations in horses and dogs.²¹⁻²³ Limited information is available on the pharmacokinetics of TA in horses.^{19,24,25}

The purpose of the study reported here was to compare the pharmacokinetics of TA in horses following IV, IA, or IM administration and to determine the pharmacodynamics of TA on the basis of changes in endogenous corticosteroid and glucose concentrations.

Materials and Methods

Horses—The study protocol was approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Six Thoroughbreds (3 male and 3 female), ranging in age from 5 to 7 years with a mean \pm SD body weight of 502.7 ± 17.8 kg, were used in the study. The horses were no longer actively racing. However, on the basis of results of direct physical examination, routine clinical biochemical analyses, and lameness examination, all horses were considered healthy and had nondiseased carpal joints. The 6 horses were assigned to 2 groups of 3; 1 group was studied at a time. Each horse in the group was administered TA IV, IM, or IA in a Latin square–assigned sequence. Grouping was based on housing, which prevented separation of groups of horse from each other for long periods and perturbations in the horses' accustomed environment that might disrupt hydrocortisone circadian rhythm.²⁶ A washout period of 4 weeks was allowed before the next drug administration.

To establish endogenous glucocorticoid circadian rhythm and daily variation in glucose plasma concentrations over a 24-hour period, each group of horses was brought into stalls 2 days before sample collection. On the third day, samples were collected each hour for 24 hours, starting at 6 AM. The 3 horses were returned to the same stalls 1 week later for the first TA administration. All horses were fed 1 quart of grain in the morning and late in the afternoon; grass hay and water were provided ad libitum.

Dose and administration—Prior to IA injection of TA into the left carpal joint or placement of a 14F catheter^a into the jugular vein for collection of blood samples, both areas were clipped of hair, washed with sterile water and surgical soap,^b and rinsed with a bactericide^c and 70% isopropyl alcohol. For IA injection into the left carpal joint, a nasal twitch was placed on each horse for restraint, the carpal joint was flexed, and TA suspension^d was injected by use of a 20-gauge needle. Successful IA injection was confirmed via visual detection of a small quantity of clear synovial fluid following slight aspiration by use of the syringe plunger. The IM injection of TA suspension was into the muscular portion of the neck. For IV administration, TA^e was

dissolved in dimethyl sulfoxide (2.0 mL) and injected into the contralateral noncatheterized jugular vein. All administrations were at a concentration of 0.04 mg/kg.

All studies started at 7 AM. Blood samples were collected prior to drug administration (0 hours) and at 2 and 5 minutes and 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48, 60, 72, 96, 120, 144, 168, 192, and 216 hours after administration. Blood samples were centrifuged ($2,500 \times g$ for 15 minutes) to obtain plasma. Aliquots of 2 mL of plasma were immediately frozen at -20°C , and within 24 hours afterward, they were stored at -70°C until analyzed. Each aliquot was used once to eliminate any effect of freeze-thaw cycles on the concentration of TA, cortisone, and hydrocortisone in the sample.

Quantification of TA, cortisone, and hydrocortisone concentrations in equine plasma—Quantification of TA, cortisone, and hydrocortisone in equine plasma was performed on the basis of described methods.²⁶⁻²⁸ All solvents^f used were of high-performance liquid chromatography grade. Because hydrocortisone and cortisone are endogenous substances, blank plasma was obtained from a horse administered dexamethasone to reduce endogenous concentrations. Then the depleted plasma was extracted 4 times by use of methyl-tert butyl ether to completely remove hydrocortisone and cortisone residues. Reference corticosteroid standard solutions for TA, cortisone, and hydrocortisone^g (10, 5, 1, 0.5, and 0.1 $\mu\text{g}/\text{mL}$ and 50 and 10 ng/mL) were prepared from each stock solution via dilution with 2mM ammonium formate-methanol (40:60 [vol/vol]; pH, 3.4) and stored at 4°C . Prepared calibrators had concentrations of 100, 50, 10, 5, 1, 0.5, and 0.1 ng/mL ; quality-control samples had concentrations of 0.5, 5, and 50 ng/mL ; and fluoxymesterone^g was the internal standard.

Analyte separation was performed by use of a C8 high-performance liquid chromatography column.^h Analyses were performed by use of a liquid chromatography–mass spectrometry system consisting of a liquid chromatography pumpⁱ with an online degasser, an autosampler,^j and a mass spectrometer^k equipped with an electrospray ionization probe.^l Standard operating procedures for the quantification of analytes by our laboratory meet requirements for accreditation by the American Association for Laboratory Accreditation and ISO 17025 International Guidelines.²⁹ The limit of quantification was 0.1 ng/mL for all analytes. The intraday precision and accuracy for a concentration range of 0.1 to 50 ng/mL were $< 5\%$ and 103%, respectively. The interday precision and accuracy were $< 6.0\%$ and 105%, respectively.

Plasma glucose was assayed by use of an automated lactate analyzer.^m Baseline samples were obtained at 7 AM prior to each drug administration.

Pharmacokinetic analysis—Plasma concentration versus time curves of TA and hydrocortisone following IV, IA, or IM administration were analyzedⁿ by use of standard nonlinear compartmental analysis.^{30,31}

Two- and 3-compartment models were fitted to the IV plasma concentration-time curve from each horse. Models that described the best fit for IV, IA, or IM administration were based on the appearance of observed

and predicted concentrations, reduction in the sums of squares, and reduction in the fractional SD of each estimated parameter (Figure 1).

The fractional rate constants for the IV compartment model were estimated with elimination (k_{10}) from the central compartment (C_1) and distribution ($[k_{1,2}$ and $k_{2,1}]$ and $[k_{1,3}$ and $k_{3,1}]$) to peripheral compartments (C_2 and C_3). Microconstants ($k_{1,2}$ and $k_{2,1}$) were converted to macroconstant exponents (alpha and beta) as described.^{32,33} A 2-compartment model with injection into the joint or muscle (C_1) with transfer from joint (k_t) or absorption (k_a) from muscle and elimination (k_{e1}) from the central com-

partment (C_2) was fitted to IA and IM administration data for each horse (Figure 1).

Indirect response models have been used in a number of studies to describe the effects of exogenous hormones on endogenous processes.^{34,35} A hydrocortisone suppression model was used in horses in the present study to describe the inhibition of the secretion of endogenous hormone hydrocortisone on the basis of the changing concentrations of TA administered IV, IA, or IM. This was designated as compartment 3 (Figure 1).

Transfer of TA (g/h) out of the joint or muscle from compartment 1 (C_1) into compartment 2 (C_2) was described by the following equation:

$$dM_1(t)/dt = -k_t \bullet M_1(t)$$

where $M_1(0) = M_10$.

The rate of change of the concentration of TA (g/L/h) in C_2 was described by the following equation:

$$d(TA_2)/dt = (k_t \bullet M_1 - k_{e1} \bullet M_2)/V_2$$

where M_10 is the quantity (μg) of TA injected into the joint or muscle (C_1), k_t is the fractional transfer rate constant (h^{-1}) from C_1 to C_2 , V_2 is the volume (L) of compartment 2 (C_2), $M_1(t)$ and $M_2(t)$ are functions describing the quantities (μg) of TA in C_1 and C_2 at time t , and k_{e1} is the elimination rate constant (h^{-1}) from C_2 . The concentration of TA in C_2 controls the pattern of change in the concentration of hydrocortisone in compartment 3 (C_3).

Suppression of hydrocortisone production attributable to plasma TA was calculated from the following equation:

$$k_{in} = ([k_{out} \bullet \text{HYD}_0] / [1 + \text{TA}_2 / \phi]) \bullet \text{HYD}_v$$

where k_{in} ($\mu\text{g/L/h}$) is the rate of production of hydrocortisone, HYD_0 ($\mu\text{g/L}$) is the baseline hydrocortisone concentration, k_{out} (h^{-1}) is the natural hormone degradation rate, TA_2 ($\mu\text{g/L}$) is the concentration of TA in C_2 (L), and ϕ ($\mu\text{g/L}$) is a parameter that influences the persistence of inhibition of hormone secretion by TA. Hydrocortisone values were converted to micrograms by use of published volume of distribution values of hydrocortisone (HYD_v).³⁶ Fixing other model parameters but allowing bioavailability to increase allowed modeling of an increase in the persistence of the inhibition of hormone secretion or extension of the duration of drug effect.^{37,38} The rate of increase or decrease of plasma hydrocortisone concentration ($\mu\text{g/h}$) was calculated from the following equation:

$$\text{Plasma hydrocortisone concentration} = (k_{in} - k_{out} \bullet \text{HYD}_0) \bullet \text{HYD}_v$$

The baseline production rate ($\mu\text{g/h}$) of hydrocortisone was calculated from the following equation setting the net rate of increase of hydrocortisone to zero:

$$k_{in} = (k_{out} \bullet \text{HYD}_0) \bullet \text{HYD}_v$$

The pharmacokinetic and pharmacodynamic data were fitted simultaneously. The IV data were modeled

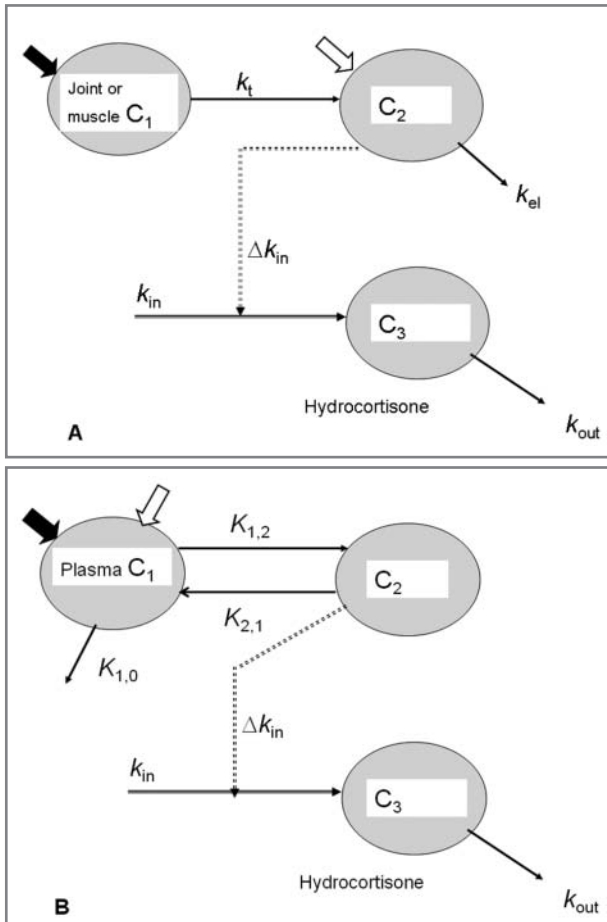


Figure 1—Two-compartmental models used in a study of pharmacokinetics of IA, IV, and IM administration of TA and its effect on endogenous hydrocortisone and cortisone in horses. A—A 2-compartment model of the rate of absorption from muscle and rate of transfer from synovial fluid of TA. Compartment C_1 is the joint synovial fluid or IM site of injection of TA with rate of transfer (k_t) to and rate of elimination (k_{e1}) from the central compartment (C_2). The black arrow represents IA or IM injection of TA, and the white arrow represents the observational sites for TA and hydrocortisone. Natural production rate of hydrocortisone is k_{in} . Notice that administration of triamcinolone perturbs (dotted line) the production rate and causes it to change (Δk_{in}). Compartment C_3 is the indirect response compartment when exposure to TA induces a pharmacological feedback mechanism altering production of hydrocortisone. Natural hormone degradation rate of hydrocortisone is k_{out} . B—A 2-compartment exchange model of IV administration of TA. The black arrow represents IV injections of TA into C_1 , and the white arrow represents observational sites for TA and hydrocortisone. C_2 is the peripheral distribution compartment following IV administration. C_3 is the indirect response compartment.

identically, with the concentration of TA in C_1 controlling the pattern of changes in the concentration of hydrocortisone in C_3 .

A number of weighting schemes ($W[K]$) were used for IV, IM, and IA data during the fitting process. Fractional SD was in the form of $W(K) = 1/(C \cdot QO[K])^2$, where $QO(K)$ is the k th observed datum and C is the fractional SD. The SD weighting scheme was $W(K) = 1/C^2$. The fractional SD weighting process favors the terminal phase of the decay curve, where SD favors the larger and intermediate data points. The fitting process (iterations) ceased when improvement in the sums of squares of the last iteration was $< 1\%$.³²

The A and B coefficients (ng/mL) for IV administration were calculated from the dose, V_c , and relevant compartmental rate constants.³⁹ Half-lives of the apparent distribution (alpha) and elimination (beta) phases were calculated as $\ln 2$ divided by the alpha and beta exponents. Plasma concentration at 0 time (C_p^0) was the sum of the coefficients A and B. The total AUC from 0 to infinity was calculated by the trapezoid method plus end area correction, which was the last plasma concentration ($C_{p_{it^*}}$) to infinity and is estimated by $C_{p_{it^*}}/k_{el}$, where k_{el} is the relevant terminal elimination rate constant for IV, IA, or IM administration.⁴⁰ Fractional drug quantity absorbed from muscle or transferred from the joint to plasma was calculated as follows:

$$AUC_{IA}/AUC_{IV} \text{ and } AUC_{IM}/AUC_{IV}$$

Volume of the central compartment following IV administration was calculated as follows:

$$V_c = D_{IV}/C_p^0$$

where D_{IV} is the dose administered IV and C_p^0 is the plasma concentration at time zero. Ratios of the inter-compartmental fractional rate constants $k_{1,2}/k_{2,1}$ multiplied by V_c were used to calculate the volume of C_2 , and the $V_{d_{ss}}$ following IV administration was calculated as the sum of all compartments⁴¹:

$$V_{d_{ss}} = ([k_{1,2} + k_{2,1}]/k_{2,1})V_c$$

Table 1—Pharmacokinetic parameter estimates following a single IV administration of TA at a dose of 0.04 mg/kg in 6 horses.

Parameter	Median (range)
C_p^0 (ng/mL)	28.1 (19.4–33.2)
A (ng/mL)	19.5 (12.5–24.7)
α (h)	1.4 (1.0–2.8)
$T_{1/2\alpha}$ (h)	0.50 (0.24–0.67)
B (ng/mL)	8.3 (5.5–12.2)
β (h)	0.11 (0.10–0.14)
$T_{1/2\beta}$ (h)	6.1 (5.0–6.4)
$AUC_{0-\infty}$ (ng/h/mL)	89.3 (63.6–103.9)
Cl (mL/h/kg)	447.8 (384.8–632.9)
V_c (L/kg)	1.4 (1.2–2.1)
V_c^c (L/kg)	2.0 (1.1–3.0)
$V_{d_{ss}}$ (L/kg)	3.3 (2.7–5.0)

$AUC_{0-\infty}$ = Area under the plasma concentration-time curve from 0 to infinity. C_p^0 = Concentration at time 0. A and B = Coefficients. α and β = Exponents. Cl = Total body clearance. V_c = Volume of compartment 2.

Clearance was calculated as follows:

$$D_{IV}/AUC_{0-\infty}$$

Statistical analysis—Pharmacokinetic parameter estimates of TA, hydrocortisone, and cortisone were expressed as median and range, and nonparametric Wilcoxon and Kruskal-Wallis rank-sum tests were used for statistical comparisons.^{42,0} An ANOVA was used for parametric analysis of plasma hydrocortisone and glucose concentrations over time and AUC comparisons. Plasma concentrations of TA were expressed as mean \pm SD. Values of $P < 0.05$ were considered significant.

Results

IV administration of TA—The plasma concentration-time curve of TA administered IV was best described by a 2-compartment model, and the concurrent changes in hydrocortisone were described by a 3-compartment model (Figures 1 and 2). Pharmacokinetic parameters were estimated (Table 1). Triamcinolone acetate was quantifiable in all horses at 36 hours. Plasma concentrations were 36.13 ± 8.62 ng/mL, 0.54 ± 0.15 ng/mL, and 0.14 ± 0.50 ng/mL at 2 minutes, 24 hours, and 36 hours, respectively. Median alpha and beta $T_{1/2}$ were 0.50 and 6.1 hours, respectively. Fractional SD for compartmental parameter estimates used in defining the IV model and concurrent changes in hydrocortisone was 0.03 ± 0.02 .

IA administration of TA—Transfer of TA from the joint (C_1) to and elimination from the central compartment (C_2) and concurrent changes in hydrocortisone were described by a 3-compartment model (Figures 1 and 2). Pharmacokinetic parameters were estimated (Table 2). Triamcinolone was quantifiable in plasma within 5 minutes following administration of TA in 3 of the 6 horses and in all horses at 0.25 hours at plasma concentrations of 0.17 ± 0.11 ng/mL and 0.30 ± 0.39 ng/mL, respectively. Triamcinolone was quantifiable in all horses at 96 hours at a plasma concentration of 0.16 ± 0.04 ng/mL. The median transfer and elimination $T_{1/2}$ were 5.2 and 23.8 hours, respectively. Fractional SD for all compartmental parameter estimates was 0.02 ± 0.01 . Fractional quantity transferred from joint to plasma was $1.0 \pm 0.2\%$ of the administered dose.

Table 2—Pharmacokinetic parameter estimates following a single IA or IM administration of TA at a dose of 0.04 mg/kg in 6 horses.

Parameter	IA	IM
$k_{1,2}$ (h)	0.14 (0.09–1.34)	1.32 (0.003–3.81)
Transfer $T_{1/2}$ (h)	5.2 (0.49–7.3)	0.52 (0.18–2.35)
$k_{2,1}$ (h)	0.03 (0.02–0.04)	0.005 (0.002–0.007)
Elimination $T_{1/2}$ (h)	23.8 (18.9–32.2)	150.2 (104.2–290.9)
$AUC_{0-\infty}$ (ng/h/mL)	82.2 (73.6–102.6)	76.6 (28.8–107.4)
Tmax (h)	10.0 (8–12)	13.0 (12–16)
Cmax (ng/mL)	2.0 (0.94–2.5)	0.34 (0.20–0.48)

Values reported as median (range).
Cmax = Maximum concentration. $k_{1,2}$ = Transfer rate constant.
 $k_{2,1}$ = Elimination rate constant. Tmax = Time to maximum concentration. See Table 1 for remainder of key.

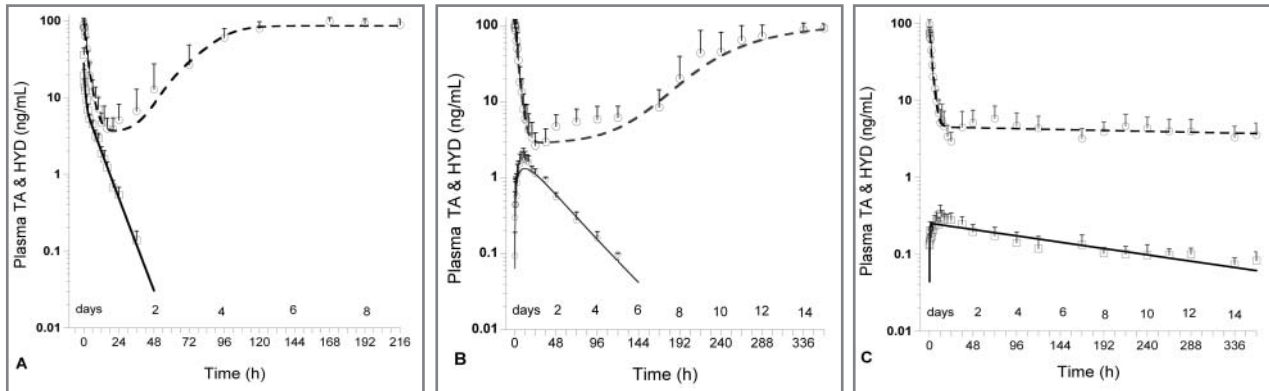


Figure 2—Mean \pm SD plasma concentrations of TA (squares) following IV (A), IA (B), or IM (C) administration (0.04 mg/kg) in 6 horses and suppression from baseline (0 hours) and recovery to baseline of the mean \pm SD plasma concentrations of endogenous hydrocortisone (HYD [circles]). Suppression and recovery of plasma hydrocortisone concentration are given relative to the changes in plasma concentration of TA. Solid and dashed lines are the best fits for plasma TA and hydrocortisone concentrations, respectively.

IM administration of TA—Absorption of TA from muscle (C_1) and elimination from the central compartment (C_2) and concurrent changes in hydrocortisone were described by a 3-compartment model (Figures 1 and 2). Pharmacokinetic parameters were estimated (Table 2). The maximum concentration following IM administration was only 0.34 ng/mL with a mean concentration of 0.19 ± 0.09 ng/mL over 360 hours (15 days). Median absorption and elimination $T_{1/2}$ were 0.52 and 150.2 hours, respectively. Fractional SD for all adjustable compartmental parameter estimates used in defining the IM model was 0.03 ± 0.02 . Fractional quantity absorbed was $0.83 \pm 0.42\%$ of the total TA administered.

Hydrocortisone—The study was conducted over a 9-month period (May through January). Baseline plasma concentrations of hydrocortisone and cortisone were 93.4 ± 19.4 ng/mL and 2.9 ± 0.63 ng/mL, respectively. Significant differences in baseline concentrations of hydrocortisone ($P = 0.02$) and cortisone ($P = 0.02$) were observed among the 6 horses, but no significant differences were observed over the study period or between sexes. The 24-hour hydrocortisone plasma concentrations of the 6 study horses had a nadir at 11 PM at 18.3 ± 8.1 ng/mL. Spikes at 7 AM and 4 PM coincided with feeding of grain. The AUC for the 24-hour hydrocortisone control period was $1,559.8 \pm 335.0$ ng/h/mL for the 6 horses. The equivalent AUC for hydrocortisone during the first 24-hour period following IV, IA, or IM administration of TA was 361.6 ± 136.7 ng/h/mL, which represented a $76.4 \pm 8.1\%$ reduction in the hydrocortisone AUC for the first 24-hour period. No significant differences were observed in the hydrocortisone 24-hour AUC between the 3 routes of administration.

Following IV, IA, or IM administration of TA, the decline in hydrocortisone plasma concentrations was significantly different from baseline within 0.75, 2, and 1 hours following IV ($P < 0.03$), IA ($P < 0.005$), and IM ($P < 0.001$) administration, respectively. Hydrocortisone plasma concentrations were still significantly different from baseline at 96 ($P < 0.001$) and 264 hours ($P = 0.006$) for IV and IA administration, respectively. Following IM administration of TA, hydrocortisone plasma concentrations had not recovered to baseline concentrations by 360 hours (15 days).

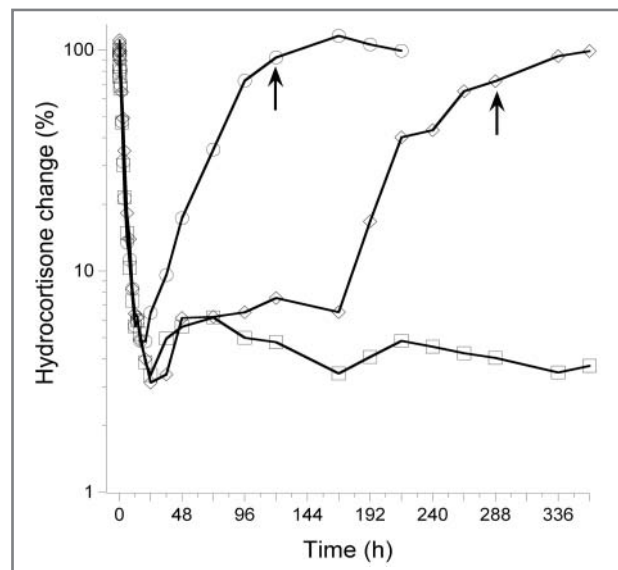


Figure 3—Percentage suppression from and subsequent recovery to baseline of plasma concentrations of endogenous hydrocortisone following IV (circles), IA (diamonds), or IM (squares) administration of TA (0.04 mg/kg) in the same horses as Figure 2. Maximum suppression of hydrocortisone occurred at 25.2 ± 7.5 hours. Following IV or IA administration of TA, hydrocortisone plasma concentrations were not significantly different from baseline at 120 and 288 hours (arrows), respectively. However, hydrocortisone plasma concentrations had not recovered to baseline by 360 hours after injection following IM administration of TA.

There were significant ($P < 0.001$) differences between plasma hydrocortisone AUC for the 360 hours after IM ($1,714.9 \pm 552.9$ ng/h/mL) or IA ($11,676.4 \pm 1,139.0$ ng/h/mL) administration. The plasma hydrocortisone AUC for IV administration at 216 hours was $11,676.4 \pm 1,139.0$ ng/h/mL. No significant differences were observed between hydrocortisone plasma AUC for IV and IA administration.

There were no significant differences in elimination of hydrocortisone (k_{out}) between IV, IA, and IM administration, but significant ($P < 0.001$) differences were noted among horses. The median k_{out} was 0.33 h $^{-1}$ (range, 0.22 to 0.65 h $^{-1}$) with a median $T_{1/2out}^{out}$ of 2.1 hours (range, 1.1 to 3.2 hours).

Following IV, IA, or IM administration, plasma concentrations of hydrocortisone declined rapidly, at

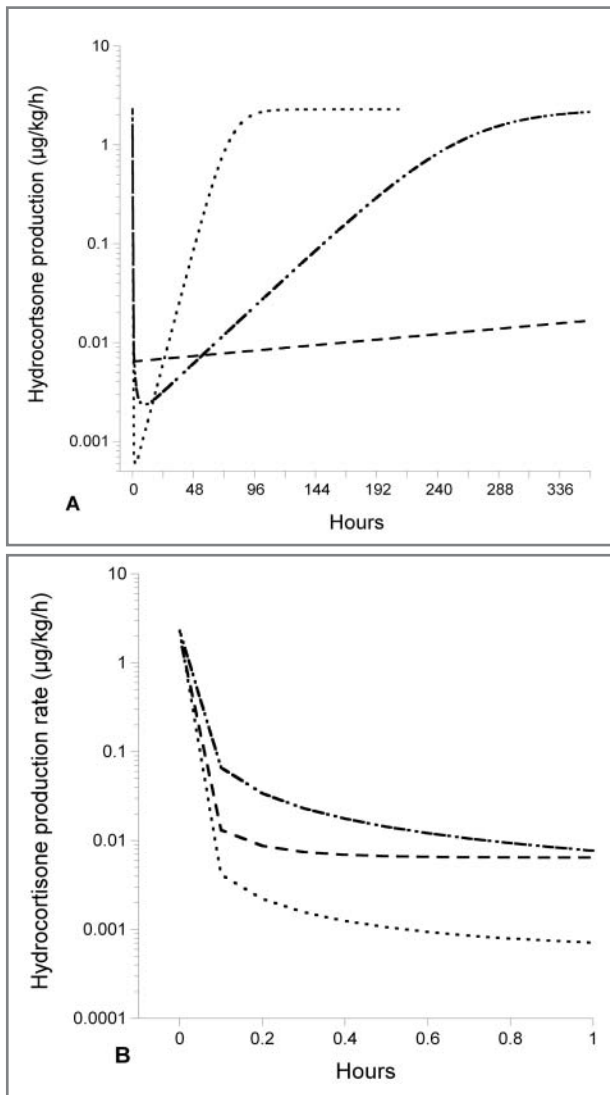


Figure 4—Suppression from baseline and recovery in the estimated production rate of hydrocortisone following IV (dotted line), IA (dotted and dashed line), and IM (dashed line) administration of TA (0.04 mg/kg) in the same horses as in Figure 2 for the whole study period (A). The rate of decline within the first hour for the 3 administrations is given (B).

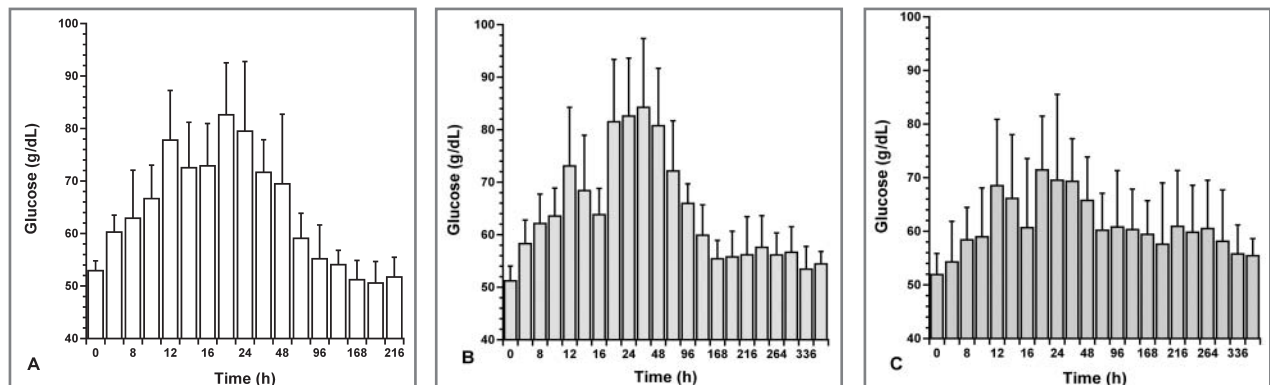


Figure 5—Mean \pm SD plasma concentrations of glucose following administration (A = IV; B = IA; C = IM) of 0.04 mg of TA/kg in the same horses as in Figure 2. Following IV administration (A), there was a significant increase in plasma glucose concentrations at 10 hours, which was significantly different from baseline at 48 hours after injection. Following IA administration (B), the increase was significantly different from baseline at 8 hours and remained so until 96 hours after injection. Following IM administration (C), the increase was significantly different from baseline at 12 hours and remained so until 48 hours after injection.

taining the lowest plasma concentration (2.7 ± 0.94 ng/mL) at 25.2 ± 7.5 hours (Figure 3). The route of administration had no effect on the hydrocortisone nadir plasma concentration and at what time it was achieved. The route of administration did have an effect on the time at which the endogenous hydrocortisone concentration recovered to baseline (Figure 2). The mean estimated baseline hydrocortisone production rate was 2.19 ± 0.56 $\mu\text{g/kg/h}$ with no significant differences in production rate among routes of administration, but as in baseline hydrocortisone concentrations, a significant ($P = 0.01$) difference was observed among horses. There was a rapid decrease in the estimated hydrocortisone production rate unrelated to the route of administration with a slow recovery to baseline that was determined by the route of administration of TA (Figure 4).

Glucose—Following IV administration (Figure 5), there was a significant ($P = 0.01$) increase in glucose concentration at 10 hours, which was still significantly ($P = 0.02$) different from baseline at 48 hours after administration. Following IA administration, the increase was significantly ($P = 0.02$) different from baseline at 8 hours and remained so until 96 hours ($P = 0.003$). Following IM administration, the increase was significantly ($P < 0.001$) different from baseline at 12 hours and remained so until 48 hours ($P = 0.005$). The increases in glucose plasma concentrations peaked at 24 hours regardless of the route of administration.

Discussion

In this study, the pharmacokinetics of TA and changes in hydrocortisone were described following IV, IA, or IM administration of 0.04 mg of TA/kg. In a previous study²⁴ in horses, the plasma pharmacokinetics of TA following IV administration of 0.2 mg/kg were described by a 3-compartment model with a terminal $T_{1/2}$ of 3 to 52 hours and detection until approximately 48 hours. The limit of quantification was 1 ng/mL for that study,²⁴ which used a higher than clinically recommended dose. In contrast, a lower dose (0.04 mg/kg) was administered in the present study and a 2-compartment model was fitted to the data with a terminal $T_{1/2}$ range of 5 to 6.4 hours. Triamcinolone acetate was

quantifiable for up to 36 hours in all horses following IV administration.

Peak plasma concentrations and elimination profiles similar to the results of the present study were reported following IA administration of 30 mg of TA. Triamcinolone serum concentration peaked at 12 hours and was quantified in serum for 78 to 102 hours in 4 horses by use of high-performance liquid chromatography.⁴³ Synovial samples were not collected in the present study because intermittent sample collection from an injected joint may have disrupted the transfer of TA from the joint to plasma. The fractional quantity transferred from joint to plasma was calculated as approximately 1.0.

In a previous study,¹⁹ plasma concentration following IM administration of 0.2 mg/kg was incompletely described because of poor absorption of TA from muscle and lack of adequate sensitivity of the method used for quantification. In the present study, the fractional quantity absorbed in 15 days was approximately 0.83% based on plasma AUC for IM administration; for IV administration, plasma AUC was based on a 36-hour plasma concentration-time curve. Plasma concentration following the IM administration peaked at 13 hours with a maximum concentration of 0.34 ng/mL, and triamcinolone was still quantifiable in plasma at 15 days after administration. This finding following IA or IM administration is explained by a so-called flip-flop phenomenon, where drug absorption is the rate-limiting step of overall drug disposition and elimination. On the basis of the marked suppression and delayed recovery of the endogenous hydrocortisone, some investigators have suggested that suppression of hydrocortisone can be taken as an indication of exogenous administration of glucocorticoid.¹⁹

Steroid receptors are asymmetric proteins, and those for glucocorticoids are found in most mammalian tissues, reflecting their ubiquitous presence and regulatory importance.⁴⁴ The administration of exogenous glucocorticoids is followed by suppression of endogenous hydrocortisone production by a feedback mechanism that inhibits the hypothalamic-pituitary-adrenal axis.^{45,46} Three major time frames of corticosteroid action have been described: fast, intermediate, and slow. The fast time frame describes a rapid effect of corticosteroids at the cell membrane inhibiting the release of corticotropin-releasing factors and stress-induced ACTH secretion. The mechanism of the intermediate time frame feedback requires the presence of a protein synthesis which is corticosteroid dependent, and the slow time frame feedback apparently involves reduction in pituitary ACTH content by decreasing concentrations of mRNA encoding the ACTH precursor molecule.⁴⁷ The immediate and prolonged suppression found in horses may be the consequence of 3 mechanisms of corticosteroid action on sensitive feedback sites.

The initial step in the development of a biological response to the administration of corticosteroids is rapid diffusion from plasma into cells for interaction with cytosolic receptors.⁴⁸ Most of these endogenous and synthetic corticosteroids are lipid soluble; they enter the target cell organs and bind to intracellular recep-

tors. Receptors have high affinity for these compounds, and low concentrations induce an effect.⁴⁹

The rapid reduction in the estimated production rate of hydrocortisone in the present study (Figure 4) indicated that the hypothalamic-pituitary-adrenal axis was rapidly suppressed, thereby shutting off production of hydrocortisone and initiating a decrease in hydrocortisone concentration. Changes in hydrocortisone production rate have also been reported in humans following dexamethasone administration.⁵⁰ The initial value is somewhat comparable to reported production rates in horses.³⁶ The change in production rate was based on measurement of the initial value and assumed a steady-state production. This production rate was determined by use of a single value obtained at the start of the present study and not by use of a mean concentration measured over a period of time that would take into account natural fluctuations in hydrocortisone production and concentrations. It is possible that the use of a mean concentration may be a more appropriate approach. The rate of change in hydrocortisone production and its response to the changing concentrations of TA are the important comparative aspects of the estimated production rate. A decrease in estimated production rates was attained in < 1 hour regardless of the route of administration.

The decrease in plasma hydrocortisone concentrations following exogenous administration of glucocorticoids was not instantaneous but occurred on the basis of the natural elimination rate of hydrocortisone following suppression of the hypothalamic-pituitary-adrenal axis. In the present study, $T_{1/2out}$ for hydrocortisone was 2.1 hours, which was similar to the elimination $T_{1/2}$ of hydrocortisone reported in horses following administration of dexamethasone (approx 1.9 hours) and methylprednisolone (approx 1.2 hours).^{17,51} Following IV administration of [³H] cortisol to horses, the $T_{1/2}$ was approximately 1.5 hours,³⁶ and following administration of hydrocortisone to humans, an approximately 1.5-hour $T_{1/2}$ has also been reported.⁵²

Other investigators have described circadian rhythm in horses that were accustomed to a management routine comprising stabling, feeding, and exercise, which may establish a circadian pattern.²⁶ Prior to drug administration, horses in the present study had a 24-hour pattern with the nadir at 11 PM at 18.3 ± 8 ng/mL. All of our studies started at 7 AM; daily samplings were also performed at 7 AM, and all changes were referenced to the morning baseline concentrations. The rapid suppression of endogenous glucocorticoids may also be related to the time of TA administration because a chronobiological sensitivity has been described in humans, with greater degree of hydrocortisone suppression detected in human patients following administration at 8 AM.⁵³

A plasma hydrocortisone concentration of approximately 0.15 ng/mL was measured within 5 minutes following IM administration of TA, indicating suppression of the hypothalamic-pituitary-adrenal axis and the production of hydrocortisone. The suppression of hydrocortisone was sustained for > 15 days; the mean plasma concentration of TA during this period was 0.19 ± 0.09 ng/mL. Results of the present study and other stud-

ies^{17,19} suggested that horses are sensitive to the effects of endogenously administered glucocorticoids. For example, dose-dependent suppression of endogenous hydrocortisone secretion had been detected in humans and a threshold concentration for maximum suppression was also suggested.² Systemic administration of 1 to 2 ng of TA/mL orally, IV, or by inhalation causes significant suppression of hydrocortisone.⁵⁴ Alternatively, a 50% inhibitory concentration of 1 ng of methylprednisolone/mL has also been reported in humans, which suggests there may be a corticosteroid-sparing dose that optimizes endogenous corticosteroid efficacy as adrenal suppression is minimized.^{55,56} In the present study in horses, no such effect was detected. Concentrations of cortisol from a single IV dose of dexamethasone recovered to baseline at 24 hours in humans,⁵⁰ compared with 96 hours in horses.⁵¹

Recovery of hydrocortisone began at approximately 19 to 36 hours following IV or IA administration, and at 15 days following IM administration, hydrocortisone suppression was still present. Slow recovery from suppression of endogenous corticosteroids may be caused by a sustained free non-protein-bound relevant tissue concentration of TA.⁵⁶

Oxidation of hydrocortisone, which occurs in the liver and kidneys, produces the inactive biomarker cortisone. Cortisone can be transformed to hydrocortisone (eg, orally administered cortisone must be metabolized to the bioactive hydrocortisone by hydroxylation of the oxygen on carbon 11 to a hydroxyl group). This conversion is an important mechanism for clearance of hydrocortisone by the kidney. Cortisone concentration was measured in this study but was not modeled. Cortisone suppression and recovery to baseline were parallel and were directly related to the changes in hydrocortisone plasma concentrations following TA administration.

Triamcinolone acetonide administered IV, IM, or IA, as with other glucocorticoids, induces a prolonged period of hyperglycemia, hyperinsulinemia, and hypertriglyceridemia. The mechanism suggested is reduced glucose use and increased gluconeogenesis.²⁴ In the present study, increases in glucose concentration occurred within 8 to 12 hours following administration and peaked at 24 hours regardless of the route of administration. For IA and IM administrations, albeit not significant, glucose concentrations were greater than baseline for the duration of the study period (Figure 5).

Results of the present study in horses indicated that a single IV, IA, or IM administration of TA had profound effects on the production of endogenous corticosteroids. This resulted in suppression to values less than baseline plasma concentrations of hydrocortisone up to 96 and 264 hours for IV and IA administration, respectively. At 360 hours following IM administration, concentrations of hydrocortisone had not recovered to baseline.

This suppression of production and concentrations of endogenous glucocorticoids lasted well beyond the analytic capabilities of current liquid chromatography-mass spectrometry methods to detect TA and other corticosteroids. The long duration of suppression may have important consequences when attempting to establish withdrawal times following administration of

synthetic glucocorticoids in racehorses if the criteria are based on concentrations that reflect the absence of measurable physiologic effects. The pharmacokinetic-pharmacodynamic model in this study describes the pharmacokinetics of TA and associated changes in plasma concentrations of endogenous corticosteroids after IV, IA, or IM administration of TA in horses.

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- a. Angiocath, Becton Dickinson, Sandy, Utah.
 - b. Chlorhexidine gluconate, 4%, Purdue Fredrick Co, Stamford, Conn.
 - c. Chlorhexidine diacetate, Fort Dodge Health, Overland Park, Kan.
 - d. Vetalog-triamcinalone acetonide suspension, Fort Dodge Health, Overland Park, Kan.
 - e. Sigma-Aldrich Corp, St Louis, Mo.
 - f. Fisher Scientific, Fair Lawn, NJ.
 - g. Steraloids, Newport, RI.
 - h. ACE 5- μ m, 2.1 X 50-mm column, Mac-Mod Analytical, Chad Fords, Pa.
 - i. Surveyor liquid chromatography pump, Thermo-Fisher Scientific, San Jose, Calif.
 - j. Surveyor autosampler, Thermo-Fisher Scientific, San Jose, Calif.
 - k. Triple Stage Quadrupole Quantum Ultra mass spectrometer, Thermo-Fisher Scientific, San Jose, Calif.
 - l. Thermo-Fisher Scientific, San Jose, Calif.
 - m. YSI Stat Plus, model 2300, Yellow Springs, Ohio.
 - n. WinSAAM Simulation, Analysis and Modeling Software, National Institutes of Health, Bethesda, Md. Available at: winsaam.com. Version 3.0.8. Accessed 2009.
 - o. JMP, version 8.0, SAS Institute Inc, Cary, NC.
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