

# Pharmacokinetics of methylprednisolone acetate after intra-articular administration and subsequent suppression of endogenous hydrocortisone secretion in exercising horses

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**Objective**—To determine the pharmacokinetics of methylprednisolone (MP) and the relationship between MP and hydrocortisone (HYD) concentrations in plasma and urine after intra-articular (IA) administration of 100 or 200 mg of MP acetate (MPA) to horses.

**Animals**—Five 3-year-old Thoroughbred mares.

**Procedures**—Horses exercised on a treadmill 3 times/wk during the study. Horses received 100 mg of MPA IA, then 8 weeks later received 200 mg of MPA IA. Plasma and urine samples were obtained at various times for 8 weeks after horses received each dose of MPA; concentrations of MP and HYD were determined. Pharmacokinetic-pharmacodynamic estimates for noncompartmental and compartmental parameters were determined.

**Results**—Maximum concentration of MP in plasma was similar for each MPA dose; concentrations remained greater than the lower limit of quantitation for 18 and 7 days after IA administration of 200 and 100 mg of MPA, respectively. Maximum concentration and area under the observed concentration-time curve for MP in urine were significantly higher (approximately 10- and 17-fold, respectively) after administration of 200 versus 100 mg of MPA. Hydrocortisone concentration was below quantifiable limits for  $\geq 48$  hours in plasma and urine of all horses after administration of each MPA dose.

**Conclusions and Clinical Relevance**—Pharmacokinetics of MP may differ among IA MPA dosing protocols, and MP may be detected in plasma and urine for a longer time than previously reported. This information may aid veterinarians treating sport horses. Further research is warranted to determine whether plasma HYD concentration can aid identification of horses that received exogenous glucocorticoids. (*Am J Vet Res* 2012;73:1453–1461)

Lameness attributable to joint injury or disease is the most common cause of reduced athletic function and performance of sport horses.<sup>1–3</sup> Intra-articular administration of medications to reduce joint inflammation and signs of pain is a routine part of the management of horses in training. Glucocorticoids are the most potent and frequently used anti-inflammatory medications for IA administration and are a mainstay in the treatment of joint disease in horses. The current level of competition, importance of safety, and public

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## ABBREVIATIONS

AUC	Area under the observed concentration-time curve
$C_{\text{max}}$	Maximum observed plasma concentration
GC	Glucocorticoid
HYD	Hydrocortisone
IA	Intra-articular
LLOQ	Lower limit of quantitation
$\lambda_z$	Elimination rate constant
MP	Methylprednisolone
MPA	Methylprednisolone acetate
$\text{SIE}_{\text{max}}$	Standard sigmoidal inhibitory maximum effect
SLOD	Screening limit of detection
$T_{\text{last}}$	Last observed quantifiable concentration in plasma
$T_{\text{max}}$	Time to maximum observed plasma concentration

scrutiny of equine athletes make knowledge of appropriate withdrawal times for medications administered IA important to ensure circulating blood concentra-

tions of those medications are below permissible limits. The United States Equestrian Federation and Fédération Equestre Internationale establish withdrawal times and SLODs for drugs in sport horses engaging in competition. To aid veterinarians treating horses in competition and those advising equestrian teams, the United States Equestrian Federation and Fédération Equestre Internationale have published guidelines that distinguish between routine, legitimate use of medications and deliberate and calculated misuse of substances or techniques to affect a horse's performance (ie, doping).<sup>3,4</sup> The purpose of establishing SLODs is to protect the welfare of horses and the integrity of equine sports.

Corticosteroids, such as MP, are approved by the United States Equestrian Federation and Fédération Equestre Internationale for IA administration to horses in competition.<sup>3,4</sup> The SLODs for GCs in blood and urine are determined on the basis of drug concentrations in unconditioned resting horses to which those drugs have been administered; SLODs are not determined on the basis of drug concentrations in sport horses in active training.<sup>3,4</sup> Horses engaging in a high level of exercise may have different rates of systemic absorption of GCs from joints, endogenous GC activities, and volumes of distribution and elimination of GCs versus those of unfit horses. Such differences may be attributable to differences in body composition of horses and increases in renal and muscle blood flow during exercise.<sup>5</sup>

Methylprednisolone is a synthetic GC formed by attachment of a 6-methyl group to prednisone. Methylprednisolone acetate is formulated as a water-insoluble suspension for IM or IA administration. This is a repository formulation, which delays systemic absorption and prolongs the duration of action of a GC.<sup>6</sup> The hypothalamic-pituitary-adrenal axis regulates endogenous secretion of cortisol, which is influenced by the circadian rhythm<sup>7-11</sup> and increased by stressors such as hypoglycemia, hypotension, surgery, injury, and exercise.<sup>12</sup> Exercise causes a transient increase in plasma cortisol concentration in horses;  $C_{\max}$  occurs 30 minutes after exercise.<sup>13</sup> Despite this transient increase in plasma cortisol concentration after exercise, horses that are consistently exercised and trained have lower baseline plasma cortisol concentrations than do untrained horses.<sup>13</sup> Endogenous and exogenous GCs have regulatory effects on function of the hypothalamic-pituitary-adrenal axis.<sup>13</sup> Exogenous administration of GCs influences the endogenous cortisol feedback loop.<sup>14</sup> Plasma HYD concentration is an indicator of endogenous cortisol production and may be suppressed by administration of small amounts of MPA to a horse. Undetectable concentrations of HYD in plasma samples may indicate that exogenous GCs have been administered to horses from which those samples were obtained. Such plasma samples could then be analyzed to detect exogenous GCs. This method of screening for exogenous GCs may be substantially less costly than performing assays to detect exogenous GCs in each plasma sample obtained from horses in competition.

Intra-articularly administered GCs have anti-inflammatory effects that are mediated by direct inhibition of nuclear factor- $\kappa$ B<sup>15</sup> and inflammatory cyto-

kines, such as interleukin-2, -4, -6, -7, and -17 and tumor necrosis factor- $\alpha$ .<sup>16</sup> Additionally, IA administration of exogenous GCs can cause transient suppression of production and plasma concentrations of endogenous cortisol for 3 to 8 days in horses.<sup>14,17</sup> These findings are similar to those for humans; IA administration of MPA to humans causes suppression of endogenous cortisol production for 7 days.<sup>18</sup> The magnitude and duration of cortisol suppression is dependent on the preparation and dose of the exogenous GC and the number of joints injected. Furthermore, administration of a dose of a GC in  $\geq 2$  joints may result in greater and longer suppression of endogenous cortisol production than administration of that dose in 1 joint.<sup>18</sup> In horses, there is slow absorption of MPA from joints and slow release of MP from the drug preparation following IA administration of MPA,<sup>14,19</sup> which may affect the magnitude and duration of suppression of endogenous cortisol production.

The purpose of the study reported here was to determine the pharmacokinetics of MP after IA administration of 2 clinically relevant doses of MPA to conditioned exercising horses and to determine the pharmacokinetic-pharmacodynamic relationships between circulating concentrations of exogenous MP and endogenous HYD. Our hypotheses were that IA administration of a high dose of MPA to exercising horses would result in plasma and urine concentrations of MP that are detectable for a longer period than they are after IA administration of a low dose of MPA, that plasma HYD concentrations would rapidly decrease following IA administration of MPA to horses, and that the duration of suppression of HYD secretion would be longer after IA administration of a high dose of MPA than after IA administration of a low dose of MPA.

## Materials and Methods

**Animals**—Five female racing Thoroughbreds (mean  $\pm$  SD age,  $3.2 \pm 1.6$  years; mean  $\pm$  SD body weight,  $478 \pm 20.6$  kg) were purchased through an agent and included in the study. An experienced examiner (ALB) performed physical and lameness examinations for each horse; horses were determined to be healthy and free of lameness. Starting 3 weeks prior to IA injection of MPA, horses were acclimated to stalls and exercised on a high-speed treadmill (ie, conditioned) 3 times/wk (Monday, Wednesday, and Friday starting at 9 AM). The exercise protocol on each day included walking (9 km/h) for 5 minutes, trotting (16 km/h) for 5 minutes, galloping (32 km/h) for 5 minutes, and walking again (9 km/h) for 5 minutes. This protocol was intended to simulate race training. The study protocol was approved by The Ohio State University Institutional Animal Care and Use Committee.

**Experimental design**—Experimental procedures were performed for all horses during the same period. Horses were exposed to 3-week conditioning, 6-week experimental (first experimental period; IA administration of 100 mg of MPA), 2-week drug washout, and 8-week experimental (second experimental period; IA administration of 200 mg of MPA) periods. This protocol was intended to ensure MP would be cleared from plasma and urine after IA administration of 100 mg

of MPA before the second experimental period during which horses received 200 mg of MPA IA. A crossover design was not used because the time during which MP was expected to be detectable in plasma and urine samples after administration of the high dose (200 mg) of MPA was anticipated to be unpredictable and prolonged and it was expected that some horses would have detectable concentrations of MP in plasma and urine samples 8 weeks after receiving this dose of MPA. This would have necessitated entry of horses into the second experimental period at different times. Plasma and urine samples were obtained from each horse during the 2-week drug washout period and assayed to determine concentrations of MP. There were no detectable concentrations of MP in these samples during that period, and all horses entered the second experimental period at the same time.

Reference ranges for plasma and urine HYD concentrations in exercising horses were determined by personnel at the testing laboratory<sup>a</sup> by use of historical control samples (unpublished data). Blood and urine samples were collected from horses and processed in a manner such that laboratory personnel were unaware of the source of samples; samples were processed in accordance with the United States Equestrian Federation's blood and urine sample collection protocol for horses in competition. This protocol had been designed to prevent tampering with samples and ensure anonymity of horses from which samples were collected. Collection of blood and urine samples included use of United States Equestrian Federation–approved urine cups,<sup>b</sup> blood separator tubes,<sup>b</sup> and sample sealant tape<sup>b</sup>; a numeric coding system was used, and samples were submitted in duplicate.

**IA administration of MPA**—A clinically relevant IA dosing protocol for MPA was determined via a survey of equine veterinarians. The protocol included IA administration of MPA in low and high motion joints to which IA medication is commonly administered.<sup>20</sup>

For each horse, 1 hind limb (right or left) was selected by use of a randomization procedure. At the start of the first experimental period (100-mg MPA protocol), MPA<sup>c</sup> (40 mg/mL) was administered IA in the tarsometatarsal (60 mg) and metatarsophalangeal (40 mg) joints of the selected hind limb of each horse. Plasma and urine samples were obtained from horses at various times for 8 weeks after IA administration of 100 mg of MPA (6-week experimental and 2-week drug washout periods); this was at least 2 weeks past the time at which MP concentrations in urine samples of horses were undetectable. After the 2-week washout period, MPA<sup>c</sup> was administered IA in the contralateral (left or right) tarsometatarsal (80 mg), metatarsophalangeal (60 mg), and metacarpophalangeal (60 mg) joints (200-mg MPA protocol), so that no joint was injected more than once during the study. Plasma and urine samples were obtained from horses at various times for 8 weeks after IA administration of 200 mg of MPA.

Day 0 of each (administration of 100 [first experimental period] or 200 mg [second experimental period] of MPA) experimental period was defined as the day on which MPA was administered IA. On day 0 of each experimental period, horses were anesthetized

for IA administration of MPA; this was intended to ensure rapid and complete injection of MPA into joints. Horses were sedated with xylazine hydrochloride<sup>d</sup> (1.1 mg/kg, IV), and anesthesia was induced with ketamine hydrochloride<sup>e</sup> (2.2 mg/kg, IV). Xylazine hydrochloride<sup>d</sup> (0.5 mg/mL), ketamine hydrochloride<sup>e</sup> (1 mg/mL), and guaifenesin<sup>f</sup> (50 mg/mL) were administered IV at a rate of 4 mL/kg/h to maintain anesthesia. Skin over the joints to be injected with MPA was shaved and aseptically prepared. All injections were performed by the same experienced clinician (ALB), and successful IA placement of needles was determined via visual detection of clear synovial fluid in needle hubs prior to administration of MPA; 20-gauge 1-inch needles were used. Synovial fluid samples (0.5 mL) were obtained from each joint immediately before administration of MPA, and WBC counts and total protein concentrations were determined. Horses were not exercised for 3 days after IA injections; then, horses resumed the treadmill exercise protocol for the duration of the study. Blood samples (16 mL) were collected from a jugular vein of each horse and placed in lithium heparin-containing tubes on days -2, -1 and 0 (immediately prior to IA administration of MPA) and 6, 12, 24, 36, 48, 72, 96, and 120 hours and 7, 8, 11, 15, 18, 25, 32, 39, 46, and 53 days after IA administration of MPA. Urine samples (120 mL) were collected via urinary catheters immediately prior to IA administration of MPA (0 hours) and 6, 12, 24, 36, 48, 72, and 96 hours and 7, 8, 11, 15, 18, 20, 22, 25, 27, 29, 32, 34, 36, 39, 41, 43, 46, 49, 50, 52, and 56 days after IA administration of MPA. Blood and urine samples collected from day 7 through the end of each experimental period were obtained between 8 AM and 9 AM. Total urine output of horses was not determined because it was not considered feasible (because of the long duration of the study and treadmill exercise of horses). Blood samples were centrifuged (1,500 × g for 10 minutes) and plasma was harvested. Urine sediment was separated by gravity at room temperature (approx 22°C; 60 minutes) and urine supernatant was collected. Plasma and urine samples were immediately frozen at -20°C until analysis. Plasma and urine samples were shipped to a laboratory<sup>a</sup> for liquid chromatography–tandem mass spectrometry analysis.

**Quantification of MP and HYD in plasma and urine samples**—High-performance liquid chromatography–tandem mass spectrometry methods were used to determine MP and HYD concentrations in plasma and urine samples.<sup>a</sup> Plasma and urine aliquots were obtained from the middle portion of sample containers and separated on a C18 column<sup>g</sup> (4.6 × 150 mm, 5 μm particle size) by use of gradient elution with 0.1% formic acid and acetonitrile mobile phases via a high-performance liquid chromatography system.<sup>h</sup> Triple quadrupole mass transitions for MP and HYD were 357.0→135.0 and 363.0→121.0 m/z, respectively.<sup>i</sup> The LLOQs of MP in plasma and urine samples were 50 pg/mL and 0.25 ng/mL, respectively; LLOQs of HYD in plasma and urine samples were 1.0 and 0.5 ng/mL, respectively.

**Pharmacokinetic and pharmacodynamic modeling and statistical analysis**—Concentration-time profiles for MP and HYD in plasma and urine samples were

generated and used for pharmacokinetic and pharmacodynamic modeling. Noncompartmental pharmacokinetic parameter estimates were initially generated with a computer software program<sup>1</sup> for plasma and urine MP concentration versus time data for each of the 5 Thoroughbreds after IA administration of 100 and 200 mg of MPA. Linear-up–log-down calculations for AUC were performed by means of 2 potential cutoff points: last observation or infinity. For compartmental pharmacokinetic analysis, 1- and 2-compartment models were evaluated for each MP and HYD plasma sample data set to determine the best fit model for further analysis. Because of a large range in values, a 1/Y weighting scheme was used to determine  $\lambda_z$  and compartmental pharmacokinetic parameters for MP concentrations. The goodness of fit for each model was assessed by evaluation of scatterplots, diagnostic variables (Akaike information criterion and Schwarz-Bayesian criterion), residual plots, and SEs of estimates. Concentration-time plots for MP indicated biphasic profiles for MP concentrations in most horses, and compartmental modeling indicated improved fits with a 2-compartment versus a 1-compartment model for some of the horses. On the basis of these criteria, a 2-compartment (plasma [compartment 2] and tissue [compartment 3]) model, with joints serving as a reservoir (compartment 1), provided the best fit.

A pharmacokinetic-pharmacodynamic model was developed to identify relationships between plasma MP pharmacokinetics and HYD concentrations. Plasma MP and HYD concentrations were evaluated with linear, direct nonlinear, and indirect nonlinear (ie, with an effect compartment) pharmacokinetic-pharmacodynamic linked models with HYD production rate or plasma HYD concentration as the effect. For nonlinear analyses, an SIE<sub>max</sub> model with HYD concentration as the effect was evaluated. Results of these models indicated a 4-compartment model would be adequate to characterize the pharmacokinetic-pharmacodynamic relationship between plasma MP pharmacokinetic parameters and HYD concentrations. Pharmacokinetic parameter estimates were therefore used as initial values in the model to estimate HYD concentrations within the fourth compartment. Differential equations were used in the combined model to describe the rates of change of MP and HYD concentrations in the various compartments:

$$dMP1/dt = -k_{12} \times MP1$$

$$dMP2/dt = k_{12} \times MP1 + k_{32} \times MP3 - k_{23} + k_{2E} \times MP2$$

$$dMP3/dt = k_{23} \times MP2 - k_{32} \times MP3$$

$$dHYD4/dt = k_{04} - (k_{4E}) \times HYD4$$

where dMP is change in MP concentration in a given compartment (1, 2, or 3), dt is change in time, MP<sub>i</sub> is MP concentration in a given compartment (where i is compartment 1, 2, or 3),  $k_{ij}$  is the rate constant for transfer between 2 compartments (where i is the first compartment [compartment 1, 2, 3, or 4] and j is the second compartment [compartment 2, 3, or 4 or excretion {E}]), dHYD4 is change in HYD concentration in compartment 4, and HYD4 is HYD concentration in

compartment 4. All rate constants are considered first order, with the exception of  $k_{04}$ , which is the zero order rate of endogenous HYD production.

The SIE<sub>max</sub> model for the relationship between MP and HYD concentrations was determined with the following equation:

$$HYD4 = HYD4_0 \times (1 - [MP2^\gamma / \{MP2^\gamma + EC_{50}^\gamma\}])$$

where HYD4<sub>0</sub> is the baseline (ie, before IA administration of MPA) HYD concentration in compartment 4, EC<sub>50</sub> is the MP concentration producing half-maximal suppression of HYD production, and  $\gamma$  is the shape factor. Model parameter estimates were generated by use of the 4-compartment model. To normalize values for comparison between experimental periods (ie, IA administration of 100 or 200 mg of MPA), HYD concentrations were expressed as a percentage relative to the baseline value during each experimental period. During baseline conditions (when dHYD4/dt = 0),  $k_{40}$  (the instantaneous HYD secretion rate) is equivalent to  $k_{4E} \times HYD4_0$ . The volume of the HYD plasma compartment was assumed to be 63.0 mL/kg.<sup>17</sup> This enabled the total HYD4 value (HYD4<sub>0</sub>) during baseline conditions to be estimated.

Plasma HYD concentrations decreased rapidly in horses after IA administration of each dose of MPA, and the plasma sampling schedule did not allow determination of a sufficient number of plasma HYD concentrations during the elimination phase to fully characterize the elimination process and accurately estimate the HYD  $\lambda_z$ . Therefore, linear regression was used to generate crude initial estimates of the HYD  $\lambda_z$  by use of a minimum of 3 HYD concentrations in plasma samples obtained during the declining phase for HYD concentration in each horse. Other authors<sup>17</sup> defined a well-characterized model for HYD plasma concentrations and included a threshold MP concentration and a HYD production rate for MP concentrations below that threshold. Although some modeling methods were based on results of that study,<sup>17</sup> plasma sampling times did not allow thorough characterization of HYD production or elimination in horses in the present study. Plasma HYD concentrations were therefore modeled as a pharmacodynamic parameter by use of MP concentrations in an SIE<sub>max</sub> direct pharmacokinetic-pharmacodynamic model (Figure 1). The relationship between MP (compartment 2) and HYD (compartment 4) concentrations was attributed to a direct effect of MP concentration on endogenous production of HYD and was consistent with the reported<sup>17</sup> pharmacokinetic-pharmacodynamic relationship between these GCs. However, HYD concentrations were estimated in the present study by use of a direct relationship (ie, no time lag) between MP concentration in compartment 2 and HYD concentration in compartment 4 via the SIE<sub>max</sub> model.

Values for quantitative, continuous measurements (eg, MP or HYD concentration) and pharmacokinetic parameters (eg, MP C<sub>max</sub>, T<sub>max</sub>,  $\lambda_z$ , T<sub>last</sub>, apparent clearance corrected for systemic availability, and apparent volume of distribution corrected for systemic availability) for samples obtained at each time point for each MPA dose were tested for a normal distribution to en-



sure appropriate use of parametric statistical methods. Pharmacokinetic and pharmacodynamic parameter estimates for each IA MPA dose were compared via a paired Student *t* test. Urine and plasma sample HYD concentrations were compared among sampling time points via repeated-measures ANOVA and between baseline and selected postinjection sampling time points via Dunn posttest analysis. Statistical analysis was performed with statistical software.<sup>k</sup> Values of *P* < 0.05 were considered significant.

## Results

All horses recovered well from anesthesia during both experimental periods and completed the study. No adverse reactions or lameness were observed after IA administration of MPA to the horses. All synovial samples collected before IA administration of MPA had total protein concentrations and WBC counts within the reference ranges (< 2.5 g/dL and < 1,000 cells/μL, respectively).

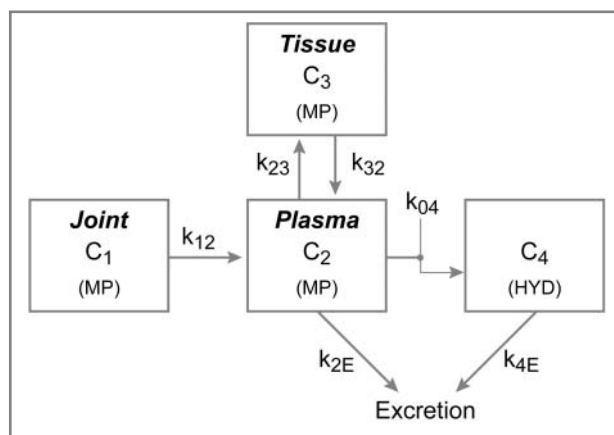


Figure 1—Combined compartmental pharmacokinetic-pharmacodynamic model for MP and HYD in horses. Compartment 1 ( $C_1$ ) represents the intra-articular reservoir into which MPA was injected, and  $K_{12}$  represents the rate constant for transfer (including conversion of MPA to MP in the joint and transfer of MP from the joint to plasma) of MP to the central plasma compartment ( $C_2$ ). Compartment 3 ( $C_3$ ) represents the peripheral tissue compartment for MP, and  $K_{23}$  and  $K_{32}$  represent the rate constants for transfer of MP between  $C_2$  and  $C_3$  and between  $C_3$  and  $C_2$ , respectively. Compartment 4 ( $C_4$ ) represents the plasma compartment for HYD,  $K_{2E}$  and  $K_{4E}$  represent the rate constants for disappearance of MP from  $C_2$  and excretion of HYD from  $C_4$ , respectively, and  $K_{04}$  represents the zero order rate of endogenous HYD production.

Table 1—Mean  $\pm$  SE noncompartmental pharmacokinetic parameter estimates for MP in plasma of 5 female 3-year-old Thoroughbreds after IA administration of 100 or 200 mg of MPA.

Parameter	100 mg of MPA	200 mg of MPA
$C_{max}$ (pg/mL)	5,070 $\pm$ 297	5,633 $\pm$ 921
$T_{max}$ (h)	12.00 $\pm$ 0	13.20 $\pm$ 3
$T_{last}$ (h)*	168 $\pm$ 13	384 $\pm$ 33
$\lambda_z$ (1/h)*	0.025 $\pm$ 0.003	0.006 $\pm$ 0.001
$t_{1/2\lambda_z}$ (h)*	30 $\pm$ 4	133 $\pm$ 27
$V_z/F$ (L)*	42,671 $\pm$ 4,545	131,258 $\pm$ 25,142
AUC (h•ng/mL)	178 $\pm$ 13	245 $\pm$ 17

\*Values are significantly ( $P < 0.05$ ) different between the 100- and 200-mg MPA IA administration protocols.

AUC = Area under the quantifiable concentration-time curve.  $t_{1/2\lambda_z}$  = Plasma half-life.  $V_z/F$  = Observed apparent volume of distribution.

**Pharmacokinetics of MP**—Noncompartmental pharmacokinetic parameter estimates for concentrations of MP in plasma were summarized (Table 1). Time until last quantifiable (ie, below the LLOQ [0.05 ng/mL] of the assay) plasma MP concentration and MP plasma half-life were significantly greater after IA administration of 200 mg of MPA than after IA administration of 100 mg of MPA. Methylprednisolone concentrations were quantifiable in plasma samples obtained from horses for a significantly longer time after IA administration of 200 mg of MPA (mean, 18 days) than they were after IA administration of 100 mg of MPA (mean, 7 days; Figures 2 and 3). No significant differ-

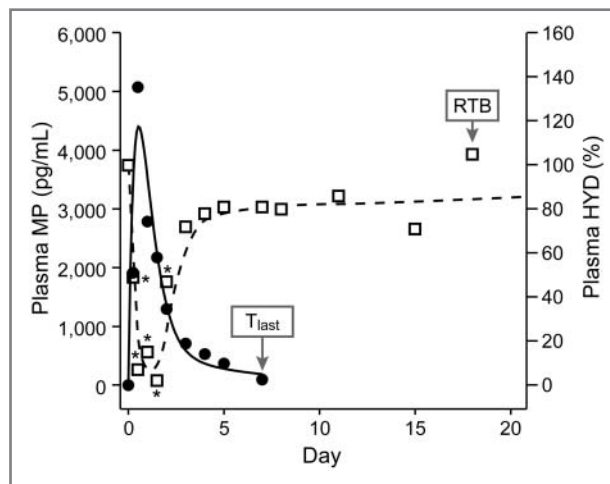


Figure 2—Mean MP (black circles; left y-axis) and HYD (white squares; right y-axis) concentrations in plasma samples obtained from 5 female 3-year-old Thoroughbreds after IA administration of 100 mg of MPA. The HYD concentrations are expressed as a percentage relative to the baseline (ie, before IA administration of MPA) value. Lines of best fit for MP (solid line) and HYD (dashed line) are indicated. The  $T_{last}$  (LLOQ, 50 pg/mL) for MP and time at which HYD concentration returned to baseline (ie, before IA administration of MPA) values (RTB) are indicated. Notice that HYD concentration rapidly decreases after administration of MPA, then increases. \*Hydrocortisone concentration is significantly ( $P < 0.01$ ) lower than the value before administration of MPA.

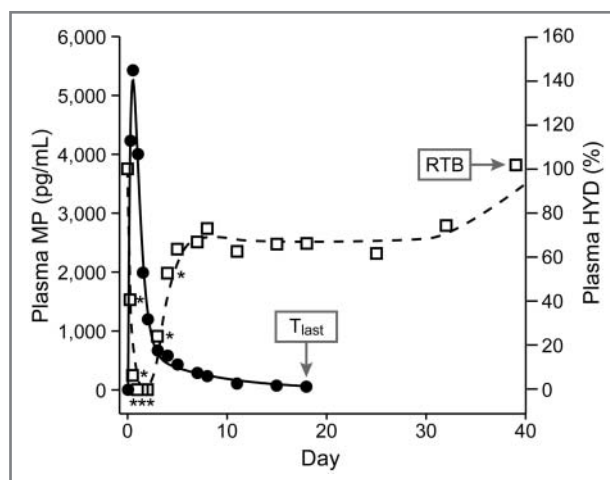


Figure 3—Mean MP and HYD concentrations in plasma samples obtained from the 5 Thoroughbreds in Figure 1 after IA administration of 200 mg of MPA. \*Hydrocortisone concentration is significantly ( $P < 0.03$ ) lower than the value before administration of MPA. See Figure 1 for remainder of key.

Table 2—Mean ± SE noncompartmental pharmacokinetic parameter estimates for MP in urine of the Thoroughbreds in Table 1 after IA administration of 100 or 200 mg of MPA.

Parameter	100 mg of MPA	200 mg of MPA
$C_{max}$ (ng/mL)*	72 ± 11	1,258 ± 259
$T_{max}$ (h)	12 ± 0	12 ± 3.3
$T_{last}$ (h)*	269 ± 27	749 ± 152
AUC (h•mg/mL)*	4.1 ± 0.3	42.7 ± 7.4

See Table 1 for key.

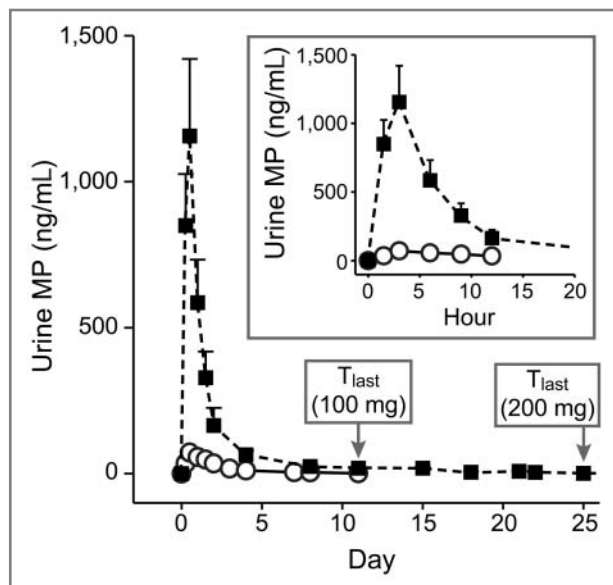


Figure 4—Mean MP concentrations in urine samples obtained from the 5 Thoroughbreds in Figure 1 after IA administration of 100 (white circles) or 200 (black squares) mg of MPA. The  $T_{last}$  for MP in urine (LLOQ, 0.25 ng/mL) after administration of 100 ( $T_{last}$  [100 mg]) and 200 mg ( $T_{last}$  [200 mg]) of MPA are indicated. The inset graph indicates values for hours 0 through 12. Notice that x-axis labels differ between the main and inset graphs.

ences were detected for  $C_{max}$  or AUC of MP in plasma after IA administration of 100 or 200 mg of MPA to horses.

For urine samples,  $C_{max}$ ,  $T_{last}$  and AUC of MP were significantly greater after IA administration of 200 mg of MPA than after IA administration of 100 mg of MPA (Table 2; Figure 4). Mean time that MPA concentration was greater than the LLOQ in urine samples after IA administration of 100 or 200 mg of MPA was 11 and 25 days, respectively. For 1 horse, urine sample MP concentrations were greater than the LLOQ through day 56 after IA administration of 200 mg of MPA.

Estimated joint (compartment 1), plasma (compartment 2), and tissue (compartment 3) MP intercompartmental transfer rate constants and the elimination rate constant for HYD (compartment 4) were not significantly different after IA administration of either dose of MPA (Table 3; Figure 1). Compartmental model mean ± SE estimated plasma MP AUC was significantly higher after IA administration of 200 mg of MPA (272 ± 24 h•ng/mL) versus after IA administration of 100 mg of MPA (211 ± 20 h•ng/mL).

**Plasma and urine HYD concentrations**—Hydrocortisone concentrations were significantly lower in

Table 3—Mean ± SE compartmental model parameter estimates for MP and HYD in the Thoroughbreds in Table 1 after IA administration of 100 or 200 mg of MPA.

Parameter	100 mg of MPA	200 mg of MPA
$V2/F \times 10^5$ (mL)*	100.26 ± 7.22	180.72 ± 19.96
$k_{12}$ (1/h)	0.08 ± 0.006	0.10 ± 0.020
$k_{21}$ (1/h)	0.05 ± 0.003	0.04 ± 0.001
$k_{2E}$ (1/h)	0.03 ± 0.007	0.03 ± 0.003
$k_{23}$ (1/h)	0.02 ± 0.003	0.009 ± 0.002
AUC (h•ng/mL)*	211 ± 20	272 ± 24
$CL/F \times 10^4$ (mL/h)*	49.33 ± 5.04	75.92 ± 6.73
$V3/F \times 10^5$ (mL)*	223.21 ± 66.24	569.44 ± 57.77
$T_{max}$ (h)	14 ± 0.9	12 ± 1.0
$C_{max}$ (ng/mL)	4 ± 0.3	5 ± 1.0
$EC_{50}$ (pg/mL)*	1,491 ± 396	371 ± 98
$\gamma$	3 ± 0.5	6 ± 2.0
$k_{4E}$ (1/h)	0.3 ± 0.04	0.7 ± 0.10

CL/F = Observed apparent clearance divided by systemic availability (assumed to be 1).  $EC_{50}$  = Plasma concentration of MP that causes a 50% decrease in plasma HYD concentration.  $k_{12}$  = Rate constant for transfer of MP from joints to plasma.  $k_{23}$  = Rate constant for transfer of MP from plasma to tissue.  $k_{32}$  = Rate constant for transfer of MP from tissue to plasma.  $k_{2E}$  = Rate constant for clearance of MP from plasma.  $k_{4E}$  = Rate constant for clearance of HYD (estimated from the slopes of the declining phases of HYD after administration of MPA).  $V2/F$  = Volume of distribution of MP in plasma.  $V3/F$  = Volume of distribution of MP in tissue.  $\gamma$  = Shape factor. See Table 1 for remainder of key.

plasma samples obtained from horses 6 hours after IA administration of either dose of MPA, compared with those in plasma samples obtained before administration of MPA. Plasma HYD concentrations were less than the LLOQ (1 ng/mL) in plasma samples obtained 6 hours after IA administration of either dose of MPA. Hydrocortisone concentrations were not significantly different from baseline concentrations in plasma samples obtained from horses from 48 hours after IA administration of 100 mg of MPA and 96 hours after IA administration of 200 mg of MPA through the end of the experimental periods. Plasma sample HYD concentrations were similar to baseline values by 18 and 39 days after IA administration of 100 and 200 mg of MPA, respectively. Urine sample HYD concentrations followed a pattern similar to that detected for plasma samples during the experiments, although values in urine samples had higher variability (data not shown).

## Discussion

The purpose of the present study was to determine the pharmacokinetic characteristics of MP after IA administration of 100 or 200 mg of MPA to Thoroughbreds and the pharmacokinetic relationship between IA MPA administration and endogenous HYD production. The higher  $T_{last}$  and longer terminal-phase half-life of MP in plasma after IA administration of 200 mg of MPA versus those after IA administration of 100 mg of MPA may be attributable to longer transfer of MP from joints into the systemic circulation of horses after receiving that higher dose. Although a significant difference was identified between model-estimated AUCs for each MPA dose, the mean ± SE AUC for the 200-mg dose of MPA (272 ± 24 h•ng/mL) was less than the expected value, considering the mean ± SE AUC for the 100-mg dose was 211 ± 20 h•ng/mL (ie, the increase in AUC was not proportional to the increase in MPA dose). Sim-

ilarly, higher MP concentrations and AUCs were detected for urine samples obtained after IA administration of 200 mg of MPA versus those in urine samples obtained after IA administration of 100 mg of MPA. This finding indicated that a greater portion of the 200-mg dose of MPA was renally excreted compared with that of the 100-mg dose of MPA. This may have contributed to the less-than-proportionally higher plasma MP concentration and AUC after administration of the 200-mg MPA dose versus those after administration of the 100-mg MPA dose.

Results of the present study differed from those of other studies. In another study<sup>17</sup> in which a single IA dose of 200 mg of MPA was administered to nonexercised horses, there was a shorter  $T_{last}$  for MP (range, 6 to 8 days) than was detected in the present study after administration of that dose of MPA to horses. Authors of another study<sup>14</sup> in which horses received a single IA dose of 100 mg of MPA reported a shorter  $T_{last}$  for MP (24 hours) than was detected in the present study after administration of that dose of MPA to horses; this finding was likely a limitation of the LLOQ for MP in that study (5 ng/mL), which was higher than the LLOQ for MP in the present study (50 pg/mL). Authors of another study<sup>19</sup> found only trace concentrations of MP in plasma after IA administration of 100 mg of MPA to horses, but synovial fluid samples obtained from joints of those horses had detectable concentrations of MPA and MP up to 6 and 39 days after IA MPA administration, respectively. The longer time that MP was detectable in plasma samples in the present study versus those other studies<sup>14,17,19</sup> was most likely attributable to the more sensitive detection techniques used, treadmill exercise of horses, and injection of MPA into multiple joints in the present study. A long-acting repository formulation of a drug (eg, MPA) would be expected to result in prolonged absorption from joints, which may have contributed to long plasma detection times for MP after administration of the high dose (200 mg) of MPA to horses in the present study. This finding indicated there may be potential for positive results of a blood drug test > 3 weeks after IA administration of a total dose of 200 mg of MPA to horses. The  $C_{max}$  of MP was higher, although not significantly, after administration of the higher dose of MPA in the present study compared with that after administration of the lower dose. This finding corresponded with the finding of rapid accumulation of MP in urine and significantly greater urine MP concentrations (on the basis of  $C_{max}$ ,  $T_{last}$ , and AUC) after administration of the higher MPA dose, compared with those after administration of the lower MPA dose to horses in the present study. Methylprednisolone was detectable in urine 4 days longer than it was in plasma after IA administration of 100 mg of MPA (11 and 7 days, respectively) and was detectable in urine 6 days longer than it was in plasma after IA administration of 200 mg of MPA (25 and 19 days, respectively). One horse had detectable concentrations of MP in urine through day 56 after IA administration of 200 mg of MPA, indicating MP concentrations in urine samples varied among horses. Methylprednisolone was detectable in urine for a longer time than it was in plasma, which suggested that a horse receiving IA MPA before competition in

accordance with established regulations and guidelines may have positive results for MP in urine > 4 weeks after the drug was administered.

Plasma HYD concentrations were lower than baseline concentrations for 2 and 4 days after IA administration of 100 or 200 mg of MPA, respectively. These findings indicated that horses with low or undetectable plasma concentrations of hydrocortisone could potentially have received exogenous GCs. Additional testing of plasma or urine samples to detect exogenous GCs would be warranted in such instances. These results supported our hypothesis that low plasma HYD concentrations could serve as a marker for systemic exposure to exogenous GCs (eg, MPA). The close relationship between IA administration of MPA and reduced plasma HYD concentrations detected in the present study and in another study<sup>17</sup> was suggestive of a cause and effect relationship. The procedures (eg, anesthesia, joint injection, and collection of blood and urine samples) that horses in these studies were exposed to increased plasma cortisol concentrations because of stress responses.<sup>13,14</sup> Inclusion of sham-injected horses in the present study may have allowed confirmation of a cause and effect relationship between IA administration of MPA and a decrease in endogenous HYD concentrations in plasma; however, such horses were not included because it was considered cost-prohibitive. Confirmation that horses not injected with exogenous GCs consistently have detectable concentrations of HYD in plasma would be necessary before determination of plasma HYD concentration could be useful as a screening tool for exposure to those drugs. Systemic exposure to exogenous GCs reduces endogenous production of HYD via effects on corticotropin-releasing hormone, which stimulates cleavage of ACTH from pro-opiomelanocortin in corticotropic cells of the anterior pituitary gland. Then, ACTH stimulates synthesis of GCs in the zona fasciculata of adrenal glands. Hydrocortisone production by cells in the zona fasciculata requires modification of progesterone by 17-hydroxylase and 21-hydroxylase. Primary routes of elimination of HYD include renal excretion and metabolism via 20-beta hydroxylase to produce cortisol. Cortisol causes an increase in blood glucose concentration, reduces muscle protein synthesis, functions as an anti-inflammatory factor, and promotes salt and water retention. Circulating cortisol concentration is regulated with a negative feedback loop via inhibition of production of corticotropin-releasing hormone in the hypothalamus and ACTH in the pituitary gland. Cortisol is eliminated via the kidneys, where it is inactivated by conversion to cortisone.<sup>21</sup> The effect of the circadian rhythm on plasma and urine cortisol concentrations in horses has been determined in several studies.<sup>7-9</sup> In the present study, plasma and urine samples collected from day 7 through the end of each experimental period were obtained between 8 AM and 9 AM to minimize effects of circadian rhythm on concentrations of GCs. Suppression of endogenous cortisol production may have important implications for establishing withdrawal times following administration of synthetic GCs. Further research is warranted to determine whether measurement of plasma HYD concentra-

tions can be used as a screening tool for identification of horses that received exogenous GCs.

Results of previous studies<sup>14,17</sup> regarding pharmacokinetics of MP and endogenous cortisol in plasma after IA administration of MPA in exercised horses are incomplete because of a lack of adequate sensitivity of the methods used for quantification. Compared with methods used in those other studies, liquid chromatography–tandem mass spectrometry is a more sensitive method for detection of GCs.<sup>22</sup> Use of that method in the present study enabled more complete determination of the pharmacokinetic characteristics of MP and their relationship to endogenous HYD production in horses than were determined in other studies.

In the present study, 2 clinically relevant protocols for IA administration of GCs were compared; these protocols were similar to those used for IA administration of GCs to sport horses.<sup>20</sup> Intra-articular administration of low doses of GCs has become common practice because the detrimental effects of GCs on cartilage are a dose-dependent event. Importantly, the present study was not designed or intended to determine biological effects of MPA in joints or to allow formulation of recommendations regarding medical use of MPA in joints. Equine practitioners differ in their preferences as to the GCs they select for IA administration; these preferences are influenced by range of motion of joints (high-motion vs low-motion joints), sport in which horses are engaged, and geographic location of the veterinarian. In the United States, it is common practice to have horses resume training within a few days after IA administration of medication and multiple joints or sites are frequently treated. Results of the present study were relevant to use of these clinical protocols and to currently recommended drug testing protocols.

The pharmacokinetic-pharmacodynamic relationships between MP and HYD were characterized by use of a direct SIE<sub>max</sub> model in the present study. Because of the plasma sampling protocol in the present study, HYD production and elimination rates were estimated with high uncertainty. Therefore, values of the MP and HYD pharmacokinetic-pharmacodynamic model parameters determined in the present study would need to be validated in future experiments prior to use for prediction of plasma HYD concentrations after administration of exogenous GCs to horses. Nonetheless, the pharmacokinetic data and values of estimated model parameters indicated there were substantial differences in pharmacokinetic characteristics of MP after IA administration of 100 or 200 mg of MPA to horses in the present study.

Population modeling of data from the present study could be performed and might provide an estimate of between-subject variability and the uncertainty associated with extrapolating these data to a population of competing sport horses. Such analyses would be interesting and might indicate a need for additional studies with greater numbers of horses to support the validity of extrapolation of data to other populations of horses. Because of the small dataset (5 horses) and experimental procedures used (treadmill exercise of horses) in the present study, extrapolation of our findings to larger populations of sport horses in competition should be performed with caution.

Results of the present study supported our hypotheses that MP would be detectable in plasma and urine samples for a longer time after IA administration of a high dose of MPA versus a low dose of MPA, that plasma HYD concentrations would rapidly decrease after IA administration of MPA, and that the duration of suppression of HYD secretion would be longer after administration of a high dose of MPA than after administration of a low dose of MPA. Further research is warranted to validate use of plasma HYD concentrations as a screening tool for identification of horses that received exogenous GCs.

- a. United States Equestrian Federation Equine Drug Testing and Research Laboratory, Ithaca, NY.
- b. Provided by the United States Equestrian Federation, Ithaca, NY.
- c. DepoMedrol, Pfizer Inc, New York, NY.
- d. Butler Animal Health Supply Co, Dublin, Ohio.
- e. Ketaset, Fort Dodge Animal Health, Madison, NJ.
- f. Vedco Inc, St Joseph, Mo.
- g. ZORBAX Eclipse XDB C18 column, Agilent Technologies, Santa Clara, Calif.
- h. Agilent 1200 HPLC system, Agilent Technologies, Santa Clara, Calif.
- i. Agilent 6410A triple quadrupole liquid chromatograph–mass spectrometer, Agilent Technologies, Santa Clara, Calif.
- j. WinNonlin, version 5.2.1, Pharsight Inc, Mountain View, Calif.
- k. SAS, version 9.2, SAS Institute Inc, Cary, NC.

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