Pharmacokinetics of methylprednisolone acetate after intra-articular administration and its effect on endogenous hydrocortisone and cortisone secretion in horses

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**Objective**—To determine the pharmacokinetics of methylprednisolone (MP) and develop a pharmacokinetic-pharmacodynamic model of the related changes in plasma concentrations of endogenous hydrocortisone (HYD) and cortisone (COR) following intra-articular administration of methylprednisolone acetate (MPA) in horses.

**Animals**—6 Thoroughbreds.

**Procedures**—In each horse, 200 mg of MPA was injected intrasynovially into a carpal joint, and plasma MP, HYD, and COR concentrations were determined via liquid chromatography-mass spectrometry.

**Results**—A 5-compartment pharmacokinetic-pharmacodynamic model was used to describe the concatenated changes in the plasma concentrations of MP, HYD, and COR and to estimate the instantaneous rate of endogenous HYD production. The median transfer half-life (t1/2) of methylprednisolone from the joint to plasma and elimination half-life (t1/2e) from plasma were 1.7 and 19.2 hours, respectively. Maximum plasma concentration of methylprednisolone was 7.26 ± 3.3 ng/mL at 8 hours, which decreased to 0.11 ± 0.08 ng/mL at 144 hours after injection. At 3 hours after MPA administration, plasma COR and HYD concentrations were significantly decreased from baseline values (from 2.9 ± 0.28 ng/mL to 2.10 ± 1.0 ng/mL and from 61.1 ± 18.9 ng/mL to 25.7 ± 12.1 ng/mL, respectively).

**Conclusions and Clinical Relevance**—The sensitivity of the analytic method used allowed complete description of the related kinetics of MP, HYD, and COR following intra-articular administration of MPA. A single intra-articular administration of MPA profoundly affected the secretion of HYD and COR in horses; secretion of endogenous corticosteroids remained suppressed for as long as 240 hours after injection. (Am J Vet Res 2006;67:654–662)

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>MP</td>
<td>Methylprednisolone</td>
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<tr>
<td>MPA</td>
<td>MP acetate</td>
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<tr>
<td>IA</td>
<td>Intra-articular</td>
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<td>HYD</td>
<td>Hydrocortisone</td>
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<td>COR</td>
<td>Cortisone</td>
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<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
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<tr>
<td>MTBE</td>
<td>Methyl-tet-butyl ether</td>
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<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
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<tr>
<td>FSD</td>
<td>Fractional SD</td>
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<tr>
<td>t1/2</td>
<td>Half-life</td>
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<tr>
<td>AUC</td>
<td>Area under the concentration curve</td>
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<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Time to maximum plasma concentration</td>
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<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Maximum plasma concentration</td>
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Methylprednisolone, a 6-methyl derivative of prednisolone, is a synthetic glucocorticoid used in veterinary and human medicine. Methylprednisolone sodium succinate formulated as a water-soluble preparation for IV administration and a water-insoluble prodrug MPA formulated as an aqueous suspension for IM or IA administration are commonly used. Methylprednisolone acetate must be hydrolyzed to the active moiety MP, and variability among species for both in vivo and ex vivo hydrolysis of various prodrugs has been discussed. The acetate form and acetamide esters are less water- and more lipid-soluble than hemisuccinate and phosphate formulations, which delay absorption and prolong their duration of action. Thus, MPA is used primarily to achieve prolonged action via IM or IA administration and is the preferred compound for IA administration. In dogs, there is slow absorption of MPA and slow release of MP following IM administration of MPA; similar findings have been reported in horses and cows following IA injections of MPA. In equine practice, corticosteroid treatment (via IA administration) is used in the management of exercise-associated articular osteoarthritis and related conditions, but its use in equine athletes remains highly controversial. The adverse effects of glucocorticoids are not minimized by IA administration; after IA administration, untoward reactions and decreased plasma concentrations of endogenous glucocorticoids for a period of 3 days in horses, as long as a 1 week in humans, and 12 weeks in cows have been reported. The purpose of the study reported here was to determine the pharmacokinetics of MP and develop a pharmacokinetic-pharmacodynamic model of the related changes in plasma concentrations of endogenous HYD and COR following IA administration of MPA in horses.
Materials and Methods

The study protocol was approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Three male and 3 female Thoroughbreds (mean ± SD age, 7.5 ± 1.8 years; mean weight, 541.0 ± 55.7 kg) were used in the study. Horses were brought into stalls 2 days before the experiment, remained housed for the duration of the study, and were fed grass hay and water ad libitum. The horses were no longer actively racing but were otherwise in good health; they were routinely dewormed and vaccinated. On the basis of findings of direct physical inspection, palpation, and a lameness examination, all horses had nondiseased carpal joints.

Dosage and IA administration of MPA—Prior to the injection of MPA into the carpal joint or placement of a 14-F catheter into a jugular vein in each horse, those areas were clipped of hair, washed with sterile water and surgical soap, and rinsed with a bactericide and 70% isopropyl alcohol. The area of catheter placement was infiltrated with a local anesthetic. The horse was halter restrained; the aseptically prepared left carpal joint was flexed, and 200 mg of MPA was injected by use of a 21-gauge needle. All injections were done by the same skilled clinician to maintain consistency, and a successful IA injection was determined through visual detection of a small quantity of clear synovial fluid prior to the injection of MPA. No adverse reactions to the injections were noted, and horses were housed in stalls for the duration of the study with daily paddock exercise. The dose of MPA injected represented 179.8 mg of MP.

All experiments started at 7 AM. Blood samples were collected prior to the IA administration of MPA (0 hours) and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48, 60, 72, 96, 144, 192, and 240 hours after IA administration. Blood samples were centrifuged (2,500 × g for 15 minutes) to obtain plasma. Aliquots of plasma (2 mL each) were immediately frozen at −20°C; within 24 hours, 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 MP, COR, or HYD in the sample.

Quantification of MP, COR, and HYD in plasma—Quantification of MP, COR, and HYD in equine plasma was based on methods devised in our laboratory. All solvents used were HPLC-grade solutions. Briefly, 100 mL of MP-free plasma was extracted 3 times with MTBE to deplete endogenous plasma COR and HYD. The organic top layer (MBTE) was discarded, and the extracted plasma was used for the preparation of calibrators and quality control samples. Reference steroid standard solutions of MP, COR, and HYD (10 µg/mL, 5 µg/mL, 1 µg/mL, 0.5 µg/mL, 0.1 µg/mL, 50 ng/mL, and 10 ng/mL) were prepared from stock solutions by dilution with ammonium formate-methanol (2mM; pH, 3.4; 40:60, vol/vol) and stored at 4°C. To prepare calibrating solutions, 10 µL of stock solution was added to 1 mL of the aforementioned extracted plasma. Concentrations of prepared calibrators were 100, 50, 10, 5, 1, 0.5, and 0.1 ng/mL, and quality control samples were 0.5, 5, and 50 ng/mL. To 1 mL of each equine plasma calibrator, quality control sample, and plasma samples obtained after MP administration, 10 µL of fluoxymesterone (10 µg/mL) as an internal standard (IS) was added. Each plasma sample was vortexed, centrifuged, and 50 µL was spotted and analyzed for MP, COR, and HYD in plasma samples obtained after MP administration.

Figure 1—Representative chromatograms (obtained via liquid chromatography-mass spectrometry) of COR, HYD, MP, and an internal standard (IS) fluoxymesterone in plasma collected from a horse after IA administration of MPA.
the internal standard was added and mixed by vortex.

Methyl-tertbutylether (5 mL) was also added to the mixture and mixed for 10 minutes on a roto-rack and then centrifuged at 2,500 × g for 15 minutes. The organic top layer was transferred to a clean glass tube by use of a Pasteur pipette or via decantation. The sample was evaporated to dryness at 45°C under a steady stream of nitrogen or air. The dried residue was reconstituted in 100 μL of mobile phase (methanol-ammonium acetate [2mM; pH, 3.4; 60:40, vol/vol]) and transferred into a 200-μL vial for analysis. Analyte separation was performed on an HPLC column (2.1 × 50 mm; 3 μm). Analyses were performed by use of liquid chromatography-mass spectrometry, involving a pump, on-line degasser, auto-sampler, and triple-stage quadrupole quantum mass spectrometer equipped with an electro-spray ionization probe. Standard operating procedures for the quantification of analytes by our laboratory meet requirements for accreditation by the American Association for Laboratory Accreditation and ISO 17,025 International Guidelines.

Retention times for MP, HYD, COR, and internal standard were 5.92, 5.33, 5.38, and 6.25 minutes, respectively (Figure 1). The LOQ was 0.1 ng/mL for all analytes. The intraday precision and accuracy was < 5% and 103%, respectively. The interday precision and accuracy were < 0% and 105%, respectively. The method is sensitive, reproducible, reliable, and fast for quantification of MP and endogenous glucocorticoids in plasma.

Pharmacokinetic analysis—Plasma concentration versus time curves of [MP], [HYD], and [COR] following IA injection of MPA were analyzed by use of standard linear compartmental analysis. By use of simulation, analysis, and modeling software, a 5-compartment model was used for simultaneous determination of the pharmacokinetics and pharmacodynamics of MP, HYD, and COR (Figure 2).

Transfer of MP out of the joint and its elimination were described by the following equations. Equation 1 describes the rate of transfer (ng/h) of MP out of the joint designated as compartment 1 (C1). This in effect describes the transfer of MP into plasma. Equation 2 describes the rate of change of the concentration of MP in plasma.

Equation 1: \[
\frac{dM_1}{dt} = -k_t \cdot M_1
\]

Equation 2: \[
\frac{d[MP]}{dt} = k_{t1} \cdot M_1 - k_{e1} \cdot M_1 \cdot V_2
\]

where \(M_1(0)\) is the quantity (ng) of MP injected into the joint (C1), \(V_2\) is the volume (mL) of compartment 2 (C2), \(M_1(t)\) and \(M_1(0)\) are functions describing the amounts of MP in C1, and \(k_t\) is the fractional transfer rate constant (h⁻¹) from C1 to C2.

Equation 3 describes the rate of change of the concentration of HYD in plasma ([HYD]). The plasma volume of a horse was estimated as 63.0 mL/kg, which was compatible with published plasma volumes of a resting horse.

Equation 3: \[
\frac{d[HYD]}{dt} = E - (k_{e3} \cdot M_3) - (k_{e4} \cdot M_3)
\]

where \(k_{e3}\) is the fractional elimination rate constant from C3, and \(k_{e4}\) is the fractional elimination rate constant from C4. The elimination of HYD following the administration of MPA is a result of direct loss (\(k_{e4}\)) and transfer of HYD to COR (\(k_{e3}\)).

The concentration of MP in C5 controls the pattern of change in the concentration of HYD in the designated compartment C5. When MP concentration in plasma is greater than the TC, HYD production is completely suppressed, but resumes when the MP concentration becomes less than the TC.

Equation 4 describes the rate of change of the concentration of COR in plasma [COR], and equation 5 represents the rate of change of the quantity of COR in tissue compartments.

Equation 4: \[
\frac{d[M_5]}{dt} = (k_{e3} \cdot M_5) - (k_{e4} \cdot M_3) \cdot V_3
\]

Equation 5: \[
\frac{dM_4}{dt} = (k_{e4} \cdot M_3) - (k_{e3} \cdot M_5)
\]
where [COR] is the plasma concentration of COR (ng/mL) at time t; M1 and M4 are the quantities (ng) of COR in compartments 4 (C4) and 5 (C5), respectively, at time t; [COR]0 is the plasma concentration (ng/mL) of COR at time zero; M1(0) and M4(0) are the quantities (ng) of COR present in C4 and C5, respectively, at time zero; k45 and k34 are intercompartmental fractional rate constants between C4 and C5; and k0 is the fractional elimination rate constant from C1. The intercompartmental microconstants for COR (k45 and k34) were converted to macroconstants α and β as previously described.16 The simulation, analysis, and modeling software used is uniquely refined to permit direct translation between the exponential and equivalent compartmental model forms. Equations 1 to 5 were translated into the software syntax by means of UF differential equation input function, and the discontinuous conditional function (E) describing the endogenous production rate of HYD was modeled by employing the software’s G 8 functional dependency equation.16

The weights W(K) applied in the fitting process used the FSD of the data and were in the form of W(K)=1/(C•QO(K)2), where QO(K) is the kth observed datum and C is its FSD.16 The fitting process (iterations) ceased when the improvement in the sums of squares of the last iteration was < 1%.

The data were fitted in a 2-step approach. The MP plasma samples from each horse were fitted and the parameters determined. The HYD and COR plasma samples were added and each horse refitted. Methylprednisolone parameters were not fixed during this fit but were allowed to float in relationship to the changes in COR and HYD.

Half-life was calculated as the natural log (base e) divided by the fractional rate constants. The total plasma MP AUC from 0 hours to last hour was calculated by use of the trapezoid rule. Time to maximum plasma concentration and Cmax were obtained directly from the experimental data. Predictions of the instantaneous production rate of HYD over time were obtained directly from the compartmental rate constants by use of previously described methods.16

Statistical analysis—Pharmacokinetic parameter estimates of MP, HYD, and COR were expressed as median (range), and the nonparametric Wilcoxon and Kruskal-Wallis rank sum tests were used for statistical comparisons.16 An ANOVA was used for parametric analysis. The plasma concentrations of MP were expressed as mean and SD. Significance was indicated at a value of P < 0.05.

Results
MP—The transfer of MP from the joint (C1) to plasma (C2) and the concurrent changes in HYD and COR were described by a 5-compartment model (Figure 2). The parameter estimates describing the total model and estimates of the pharmacokinetic parameters for MP were calculated (Tables 1 and 2). The increase and subsequent decrease of the plasma concentration-time curve of MP was plotted (Figure 3). The Cmax of MP was 7.3 ± 3.3 ng/mL, which occurred at a Tmax of 8.3 ± 0.8 hours. At 144 hours after IA injection of MPA, MP was quantifiable in all horses and, the mean ± SD plasma MP concentration had decreased to 0.11 ± 0.08 ng/mL. Methylprednisolone was still quantifiable in 2 horses at 192 hours (0.06 and 0.15 ng/mL) and in 1 horse at 240 hours (0.06 ng/mL). The FSD of all the MP pharmacokinetic-pharmacodynamic parameters for individual horses was 0.02 ± 0.01.

HYD—Estimates of the pharmacokinetic parameters for HYD were calculated (Table 3). There was an initial significant increase in the plasma concentration of HYD at 0.25 hours (P < 0.001) and 0.5 hours (P < 0.021) from a baseline value of 61.1 ± 18.9 ng/mL to 91.7 ± 16.7 ng/mL and 79.6 ± 22.4 ng/mL, respectively (Figure 4). The plasma HYD concentration subsequently returned to baseline value and continued to decrease to a concentration of 25.7 ± 12.1 ng/mL at 3 hours after IA administration of MPA; this concentration was significantly (P < 0.024) different from the baseline value. The plasma concentration of HYD decreased below the LOQ between 6 and 8 hours but returned to above LOQ in all horses between 48 and 72 hours. At 192 hours after MPA injection, the plasma HYD concentration (42.1 ± 9.2 ng/mL) was still significantly (P < 0.028) different from baseline value; at 240 hours, the concentration was 52.1 ± 9.2 ng/mL, which was not significantly (P < 0.091) different from baseline value. The initial estimated instantaneous production rate for HYD was 2.66 ± 0.84 μg/kg/h, and the final rate of HYD production was 1.42 ± 0.67 μg/kg/h (Figure 5). The FSD of all the HYD pharmacokinetic-pharmacodynamic parameters for individual horses was 0.03 ± 0.02.

COR—Estimates of the pharmacokinetic parameters for COR were calculated (Table 3). There was a slight but insignificant increase in the plasma COR

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter</th>
<th>Median estimate (range)</th>
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<tbody>
<tr>
<td>MP</td>
<td>k1 (h⁻¹)</td>
<td>0.399 (0.152–0.624)</td>
</tr>
<tr>
<td></td>
<td>k2 (h⁻¹)</td>
<td>0.038 (0.020–0.063)</td>
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<tr>
<td></td>
<td>V2 (mL/kg)</td>
<td>47.1 (29.0–58.8)</td>
</tr>
<tr>
<td>HYD</td>
<td>k30 (h⁻¹)</td>
<td>0.524 (0.335–0.911)</td>
</tr>
<tr>
<td></td>
<td>k31 (h⁻¹)</td>
<td>0.029 (0.013–0.067)</td>
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<td></td>
<td>φ (mL/h)</td>
<td>290.6 (175.7–1174.3)</td>
</tr>
<tr>
<td></td>
<td>TC (ng/mL)</td>
<td>2.7 (0.81–2.5)</td>
</tr>
<tr>
<td>COR</td>
<td>k40 (h⁻¹)</td>
<td>0.549 (0.410–1.154)</td>
</tr>
<tr>
<td></td>
<td>k45 (h⁻¹)</td>
<td>0.119 (0.056–0.281)</td>
</tr>
<tr>
<td></td>
<td>k50 (h⁻¹)</td>
<td>0.045 (0.021–0.106)</td>
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k1 = Fractional transfer rate constant from compartments 1 to 2. 
k2 = Fractional elimination rate constant from compartment 2. 
V2 = Apparent volume of compartment 2. 
k30 = Fractional elimination rate constant from compartment 3. 
k31 = Fractional elimination rate constant from compartment 4. 
k40 = Fractional elimination rate constant from compartment 4. 
k45 = Intercompartmental fractional rate constants between compartments 4 and 5. 
k50 = Intercompartmental fractional rate constants between compartments 5 and 4.

Table 2—Pharmacokinetic parameter estimates (median and range) determined by use of a 5-compartment pharmacokinetic-pharmacodynamic model to describe the transfer of MP from joints to plasma and the concatenated changes in plasma concentrations of HYD and COR in 6 horses after IA injection of MPA (200 mg).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median estimate (range)</th>
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<tbody>
<tr>
<td>k1 (h⁻¹)</td>
<td>0.399 (0.152–0.624)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>1.7 (1.4–4.5)</td>
</tr>
<tr>
<td>k0 (h⁻¹)</td>
<td>0.038 (0.020–0.063)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>19.2 (9.9–33.5)</td>
</tr>
<tr>
<td>AUCₘₘ (ng•h•mL⁻¹)</td>
<td>2175 (108.2–284.2)</td>
</tr>
</tbody>
</table>

k1 = Transfer rate constant of MP from joint to plasma. 
t1/2 = Elimination rate constant of MP from plasma. 
k0 = Elimination rate constant of MP from plasma. 
AUCₘₘ = Area under the curve (0 to 144 hours).
concentrations between 0.25 and 0.50 hours after MPA injection. Thereafter, plasma COR concentration decreased significantly ($P < 0.005$) from a baseline concentration of 2.85 ± 0.28 ng/mL to 2.1 ± 1.0 ng/mL at 3 hours after MPA injection (Figure 6). The relationship between the increase and decrease in the plasma concentration of MP and the subsequent increase in the plasma concentration of COR was illustrated graphically (Figure 3).

Plasma COR concentrations decreased to < 1 ng/mL between 6 to 8 hours following the IA injection of MPA. Plasma COR concentration increased to > 1 ng/mL between 48 and 72 hours, but at 144 hours, it was only 1.85 ± 0.57 ng/mL (which was still significantly [$P < 0.002$] lower than the baseline concentration). However, by 192 and 240 hours, the plasma concentrations of COR were 2.40 ± 0.53 ng/mL and 2.55 ± 0.39 ng/mL, respectively, and these concentrations were not significantly ($P < 0.084$ and $P < 0.28$) different from the baseline plasma COR concentration. The FSD of all the COR pharmacokinetic-pharmacodynamic parameters for individual horses was 0.04 ± 0.02.

Hydrocortisone production began to increase at 35.5 ± 7.7 hours after MPA injection, when the mean threshold plasma MP concentration was 2.4 ± 0.71 ng/mL. The COR and HYD nadir concentrations at this time (estimated from the model) were 0.16 ± 0.05 ng/mL and 1.2 ± 0.55 ng/mL, respectively.

**Discussion**

Previous descriptions of the plasma pharmacokinetics of MP and the endogenous glucocorticoids after IA administration of MPA in horses were incomplete.
because of the lack of adequate sensitivity of the methods used for quantification. In the present study, the use of highly sensitive liquid chromatography-mass spectrometry with LOQ of 100 pg/mL for all glucocorticoid analytes allowed a more complete description of the pharmacokinetics of MP and endogenous glucocorticoids following IA injection of MPA.

In the present study, plasma MP concentration peaked approximately 8 hours following the administration of MPA and was still quantifiable in horses between 144 and 192 hours (6 and 8 days). In contrast, in previously reported studies in horses, the analytical sensitivity of 2 to 3 ng/mL via HPLC allowed the measurement of MP in plasma for only as long as 8 hours following IV administration of MP. In horses, only trace concentrations of MP were detected in plasma following IA injection of MPA, but in samples taken from the joint, MP was detected for as long as 39 days and MPA was detected for 3 to 6 days after administration. In a later study, the median terminal t₁/₂ for synovial MPA was 10.3 hours and the t₁/₂ for synovial MP was 9.9 to 32.1 hours. When a radioimmunoassay technique was used to detect MP in the serum of humans with rheumatoid arthritis, peak concentrations were detected between 2 and 12 hours after IA administration; serum concentrations of MP were also detected for as long as 7 days. In the present study, synovial samples were not collected because intermittent sample collection from an injected joint may have disrupted the transfer of MP from the joint to plasma.

Following the IV administration of MP dissolved in dimethyl sulfoxide, a terminal elimination t₁/₂ of approximately 2.8 hours was reported. Rapid elimination of MP following IV administration has also been reported in humans. Intravenous administration was not performed in our study, but previous reports of the rapid elimination of MP following IV administration suggested a flip-flop model of elimination, in which the elimination was dictated by the rate of transfer of MP from synovial fluid to plasma. Other investigators have suggested that the limiting step for the transfer of MP from the joint to plasma was the slow release of MP from MPA.

Administration of synthetic or naturally occurring glucocorticoids inhibits the secretion of corticotrophin-releasing factors and arginine vasopressin and stress-induced ACTH secretion. Corticosteroid feedback inhibits the brain-hypothalamo-pituitary axes of the adrenocortical system. Endogenous corticosteroids may have their primary actions at brain and hypothalamic sites, and synthetic glucocorticoids that do not bind to transcortin may act primarily on corticotropes and regions of brain outside the blood-brain barrier. This inhibition at the level of the pituitary gland and hypothalamus is mediated through a negative feedback mechanism that controls secretion and synthesis of endogenous glucocorticoids. Others have suggested direct inhibition of cortisol secretion by the adrenal cortex, without the involvement of ACTH and the central hypothalamic-pituitary axis.

In horses of the present study, a significant increase in plasma HYD concentration and a slight increase in plasma COR concentration were detected at 0.25 and 0.5 hours following IA injection of MPA. Jugular catheters were placed prior to the start of experiments, and blood samples had been collected in the past from all horses in our study; all blood samples were carefully collected to avoid exciting the horses. Nevertheless, these initial increases in plasma HYD and COR concentrations were probably a result of the IA injection, despite the fact that only halter restraint was necessary to flex and inject the joint and that the injection was performed by a skilled clinician.

In our study, compared with baseline concentrations, both plasma HYD and COR concentrations were decreased significantly at 3 hours after MPA injection and returned to baseline values between 192 and 216 hours (8 and 9 days). The t₁/₂ values for COR and HYD in our study (approx 1.0 and 1.2 hours, respectively) were similar to those previously reported in humans and horses.

The initial step in the development of a biological response to the administration of corticosteroids is the rapid diffusion from plasma into cells for interaction with cytosolic receptors. Most of these endogenous and synthetic corticosteroids are lipid soluble; they enter the target cell organs and bind with intracellular receptors. Receptors have high affinity for these compounds, and very low concentrations produce an effect. Results of a study in dogs have suggested that a time-average feedback operates over a short time course of 30 to 60 minutes. The effects of IV administration of synthetic corticosteroid suggest that the feedback mechanism may be related to the rate at which the compound is delivered to the CNS—there appears to be a rapid feedback mechanism following an IV dose, compared with a slower feedback mechanism and suppression of endogenous glucocorticoid secre-
tion following IA, PO, or IM administrations. In the present study, the cessation of production and the subsequent decrease in plasma concentrations of HYD and COR after IA administration of MPA occurred at a later time and a higher drug concentration than that reported after IV administration of dexamethasone. This may be related to the slower increase in the plasma concentration of MP and its lower potency, compared with dexamethasone.

The high sensitivity of the analytical methods used in our study allowed a thorough description of the pharmacokinetics of MP after IA injection of MPA in horses. The model described the pharmacokinetics of MP and the concatenated changes in the plasma concentrations of endogenous HYD and COR. Despite the sensitivity of the method, quantification was limited to 6 days in all horses and beyond 6 days in only 3 horses. The terminal plasma concentrations suggested the initiation of a second compartment, and thus a second compartment was added to the model. The parameters describing this compartment were not well defined; therefore, it was not included and the model for MP reported here was selected. Such pharmacokinetic-pharmacodynamic models may be useful because they allow some understanding and predictions of important pharmacodynamic effects of different doses and dosing regimens.

In the present study, the instantaneous HYD production rates prior to and during the changes in plasma MP concentration were determined; initial and final estimated production rates were approximately 2.6 and 1.4 $\mu$g/kg/h, respectively. The initial value is somewhat comparable to a reported production rate of 2.6 and 1.4 $\mu$g/kg/h in fit horses. Other values reported in horses were much higher. The lower production rate measured in our study may be related to a difference between fit and unfit horses, the method of estimation, or residual effects of MPA administration. The change in production rate was based on the measurement of the initial value, which was an instantaneous value obtained at the start of the study and not a mean concentration measured over a period of time that would take into account natural fluctuations in HYD production; it is possible that use of a mean concentration may be a more appropriate approach.

Our model differs from previously published models in the manner in which we describe the suppression of HYD production following the administration of MP. In our simple inhibition function, we used a threshold value concentration of MP above which HYD production ceased and below which HYD production rate increased in a linear fashion. This is in contrast to other models that used inhibition functions containing a parameter related to the concentration of MP that causes a 50% inhibition in the production rate of glucocorticoid. In our approach, MP and both HYD and COR were included in a single 5-compartment model that related changes in endogenous glucocorticoid concentrations to changes in plasma MP concentration. Furthermore, the model enabled estimation of the instantaneous production rate of HYD and the time course of the recovery of plasma concentrations of HYD and COR following IA administration of MP as well as the threshold concentration of MP at which HYD production returned.

Findings of the present study point to a system that is highly sensitive to changes in exogenously administered glucocorticoids. The cessation of production of HYD and the subsequent decrease in plasma HYD and COR concentrations were rapid, but the recovery of endogenous HYD and COR to baseline concentrations was slow. By use of our model, we determined that the gradual increases in plasma concentrations of HYD and COR were a result of the slow increase in production of HYD (and concomitant conversion to COR) as plasma concentrations of MP gradually decreased to values that were far below the LOQ. Although our controlling concentration was that of MP in plasma, the suppression and recovery phase may be governed by the related concentrations of MP in peripheral compartments.

The effect of circadian rhythm on plasma cortisol concentrations has been reported in several studies. Other investigators described circadian rhythm in horses that were accustomed to management routines that included stabling, feeding, and exercise that may have entrained a circadian pattern. On the basis of this assumption, our studies were started at 7 AM, daily samplings were also conducted at 7 AM, and all changes were referenced to the baseline morning concentrations. The decrease in the endogenous corticosteroids occurred within 3 hours of the IA injection of MPA, which may have overridden any reductions associated with circadian rhythm over the immediate 12 hours and the following days. Baseline plasma concentrations of the glucocorticoids have also been measured in horses via HPLC, radioimmunoassay, radioostereosassay, and fluorometric methods.

The methods used in earlier studies measured plasma cortisol concentration only and did not distinguish between HYD and COR. This may explain the total suppression of HYD production identified in our study, compared with other reports. Hydrocortisone is the main metabolically active and naturally occurring corticosteroid and is derived from cholesterol through the progesterone metabolic pathway. The oxidation of HYD, which occurs in the liver and kidneys, produces the inactive COR. Cortisone can be transformed back to HYD; for example, orally administered COR must be metabolized to the bioactive HYD by the transformation of the oxygen on carbon 11 to a hydroxylated moiety. Conversion of HYD to COR is an important mechanism for the clearance of HYD from the body by the kidneys.

Methylprednisolone acetate and other synthetic corticosteroids used primarily for treatment of inflammation and arthritis have wide-ranging effects that include glucose metabolism, suppression of the immune system, changes in behavioral activity, and alteration of the secretion of endogenous corticosteroids.

Results of the present study in horses indicated that a single IA injection of MPA had profound effects on the production of endogenous corticosteroids and that production was not normalized for at least 240
hours after MP administration. This suppression of production outlasts the ability of current sensitive analytic methods to measure residual concentrations of MP in plasma. In our study, the plasma concentrations of MP after IA injection of 200 mg of MPA were quantifiable in all horses for up to 144 hours and in 1 horse for up to 196 hours. The long duration of the suppression of endogenous glucocorticoids may have important consequences when attempts are made to establish withdrawal times following the administration of synthetic glucocorticoids, if the criteria are based on concentrations that reflect the absence of measurable physiologic effects. The pharmacokinetic-pharmacodynamic model presented in this report describes the pharmacokinetics of MP and the concatenated changes in the plasma concentrations of endogenous corticosteroids after IA administration of MP in horses.

a. Angiocath, Becton-Dickinson, Sandy, Utah.
b. Chlorhexidine gluconate 4%; Purdue Frederick Co, Stamford, Conn.

c. Chlorhexidine diacetate, Fort Dodge Health, Fort Dodge, Iowa.

d. Lidocaine HCl 2%, Vedco, St Joseph, Mo.


g. Steraloids, Newport, RI.

h. Sigma-Aldrich, St Louis, Mo.

i. Ace GC column, Mac-Mod Analytical, Chad Fords, Pa.

j. LC/TSQ-MS/MS, Thermo Electron Corp, San Jose, Calif.


l. Reference.

m. JMP, version 4.0, SAS Institute Inc, Cary, NC.

References


