

## Disposition of methylprednisolone acetate in plasma, urine, and synovial fluid following intra-articular administration to exercised thoroughbred horses

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Knych, H. K., Harrison, L. M., Casbeer, H. C., McKemie, D. S. Disposition of methylprednisolone acetate in plasma, urine, and synovial fluid following intra-articular administration to exercised thoroughbred horses. *J. vet. Pharmacol. Therap.* 37, 125–132.

Methylprednisolone acetate (MPA) is commonly administered to performance horses, and therefore, establishing appropriate withdrawal times prior to performance is critical. The objectives of this study were to describe the plasma pharmacokinetics of MPA and time-related urine and synovial fluid concentrations following intra-articular administration to sixteen racing fit adult Thoroughbred horses. Horses received a single intra-articular administration of MPA (100 mg). Blood, urine, and synovial fluid samples were collected prior to and at various times up to 77 days postdrug administration and analyzed using tandem liquid chromatography-mass spectrometry (LC-MS/MS). Maximum measured plasma MPA concentrations were  $6.06 \pm 1.57$  at 0.271 days (6.5 h; range: 5.0–7.92 h) and  $6.27 \pm 1.29$  ng/mL at 0.276 days (6.6 h; range: 4.03–12.0 h) for horses that had synovial fluid collected (group 1) and those that did not (group 2), respectively. The plasma terminal half-life was  $1.33 \pm 0.80$  and  $0.843 \pm 0.414$  days for groups 1 and 2, respectively. MPA was undetectable by day  $6.25 \pm 2.12$  (group 1) and  $4.81 \pm 2.56$  (group 2) in plasma and day 17 (group 1) and 14 (group 2) in urine. MPA concentrations in synovial fluid remained above the limit of detection (LOD) for up to 77 days following intra-articular administration, suggesting that plasma and urine concentrations are not a good indicator of synovial fluid concentrations.

(Paper received 25 February 2013; accepted for publication 20 June 2013)

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### INTRODUCTION

Intra-articular administration of corticosteroids is commonplace in performance horses both for the treatment of exercised induced joint damage as well as prophylactically to minimize damage brought on by intensive exercise (Auer & Fackelman, 1981; McIlwraith, 1982; Genovese, 1983; McIlwraith, 1989). Damage to joints leads to release of pro-inflammatory mediators that if left unchecked can cause permanent damage (McIlwraith, 1982). Corticosteroids are potent anti-inflammatory agents that decrease the production of these pro-inflammatory mediators, thereby decreasing the acute inflammatory effects and the long-term detrimental effects. Because of the potential for corticosteroids to mask injury that may otherwise prevent a horse from competing, use of this class of drugs is

closely controlled by horse-related regulatory organizations. For example, corticosteroids are designated as Class 4 (Penalty Class C) foreign substances by the Association of Racing Commissioners International.

While its use remains somewhat controversial, methylprednisolone (MP) is one of the more commonly used corticosteroids in performance horses. MP is the 6-methyl derivative of prednisolone and has an anti-inflammatory potency 4–5× that of the endogenous corticosteroid, hydrocortisone (Ferguson *et al.*, 2009). Arguably, the most commonly used MP formulation for intra-articular administration in the horse is methylprednisolone acetate (MPA), a slightly soluble ester of MP. The acetate formulation increases the lipid solubility of the compound, providing a prolonged local anti-inflammatory effect, which is at least partly a result of increased residence time

within the joint. The anti-inflammatory effects of MPA are attributable to MP, and therefore, activity of this formulation is dependent upon cleavage of the acetate moiety by esterases present within the joint (Ferguson *et al.*, 2009).

The plasma pharmacokinetics of intra-articular MPA has been described previously (Autefage *et al.*, 1986; Lillich *et al.*, 1996; Soma *et al.*, 2006; Menendez *et al.*, 2012). In all studies, plasma concentrations of MP remained low throughout the collection period; however, the detection window varies between studies, which can be problematic when attempting to regulate this drug prior to competition. Of additional concern for drugs, such as MPA, which are administered as long-acting depot formulations, is that the low concentrations measured in plasma do not necessarily reflect the true concentration at the site of effect (the joint), suggesting an effect long after drug can be regulated. Autefage *et al.* (1986) and Lillich *et al.* (1996) report a longer MP detection window in the joint relative to plasma following intra-articular administration. In both studies, the sensitivity of the analytical assay (Autefage *et al.* (1986): 2–3 ng/mL in plasma and 10–20 ng/mL in; Lillich *et al.* (1996): 2.5–10 ng/mL) was much less than that currently available, and it is likely that MP may be present for even longer in both plasma and synovial fluid than reported in the earlier studies.

The primary goal of this study was to extend current knowledge with respect to the disposition of MP in plasma, urine, and synovial fluid following intra-articular administration in the horse. A secondary goal was to relate MP plasma and synovial fluid concentrations following intra-articular administration of MPA to exercised horses.

## MATERIALS AND METHODS

### *Horses and drug administration*

Sixteen healthy exercised to racing fit adult Thoroughbred horses including eight geldings and eight mares (3–7 years of age) were studied. Prior to and throughout the course of the study, horses were exercised 5 days a week. The general exercise protocol for these horses consists of 3 days/week on an Equineciser (Centaur Horse Walkers Inc, Mira Loma, CA, USA) (5 min at walk; 15-min trot; 5-min walk) and 2 days/week on a high-speed treadmill (Mustang 2200; Graber AG, Fahrwangen, Switzerland) (5 min at 1.5 m/s; 5 min at 4 m/s; 2 min at 7 m/s; 1 min at 9 m/s; 5 min at 1.5 m/s). Horses were not exercised on the day of or the day after synovial fluid collection. Two days after synovial fluid collection, horses were allowed to freely exercise in a round pen.

Before beginning the study, horses were determined healthy by physical examination, complete blood count, and a serum biochemistry panel that included aspartate aminotransferase, creatinine phosphokinase, alkaline phosphatase, total bilirubin, sorbitol dehydrogenase, blood urea nitrogen, and creatinine. Blood analyses were performed by the Clinical Pathology Laboratory of the William R. Pritchard Veterinary Medical Teaching Hospital of the University of California, Davis, using standard

protocols. Horses did not receive any other medications for at least 2 weeks prior to commencement of the study. This study was approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

### *Instrumentation and drug administration*

A 14-gauge catheter was placed in the external jugular vein for sampling. Each horse was weighed immediately prior to drug administration. For drug administration, the area over the antebrachio-carpal joint was scrubbed with chlorhexidine solution (Agri Laboratories Ltd, St Joseph, MO, USA) and 70% isopropyl alcohol, the joint flexed, and a total dose of 100 mg (3 mL) of MP acetate (Pfizer Animal Health, Kalamazoo, MI, USA) administered aseptically into the right antebrachio-carpal joint. The administered dose for this study was chosen based on the highest administered dose reported from a survey of racetrack practitioners.

### *Sample collection: blood, synovial fluid, and urine*

Blood samples for drug concentration determination were collected at time 0 (prior to drug administration) and at 15, 30, and 45 min, and 1, 2, 3, 4, 5, 6, 8, 12, 18, 24, 36, 48, 60, 72, and 96 h postadministration. Subsequent samples were collected on day 6, 7, 10, 13, 14, 16, 17, 21, 24, 28, 30, 35, 38, 42, and 44 postadministration. Blood samples were tested to ensure that MP was no longer detected prior to termination of sample collection. Prior to drawing each sample of blood for analysis of drug concentrations, 10 mL of blood was aspirated and discarded from the catheter and T-Port extension set (combined internal volume <2 mL). The catheter was flushed with 10 mL of a dilute heparinized saline solution (10 IU/mL) following each sampling time. Catheters were removed following collection of the 24-h sample, and the remaining samples collected by direct venipuncture. Blood samples were collected into EDTA blood tubes (Kendall/Tyco Healthcare, Mansfield, MA, USA) and stored on ice until centrifugation at 3000 *g* for 10 min at –4 °C. Plasma was then immediately transferred into storage cryovials (Phenix Research Products, Chandler, NC, USA) and stored at –20 °C until analysis (approximately 2 weeks following collection of the final sample for each administration route).

Synovial fluid was collected from eight of the sixteen horses. Prior to collection of synovial fluid, the area over the right and left carpi was scrubbed with chlorhexidine solution. Immediately prior to collection, the area over the joints was wiped multiple times with alcohol. Synovial fluid samples were collected from the right and left antebrachio-carpal and middle carpal joints by aspiration with a 21-G 1½-inch needle at 12, 24, 48, 72, 96, and 120 h postdrug administration. Additional synovial fluid samples were collected once a week starting on day 7 until 77 days postadministration. Synovial fluid samples were tested to ensure that MP was no longer detected prior to termination of sample collection. Synovial fluid was separated into aliquots in storage cryovials (Phenix Research Products) and

stored at  $-20^{\circ}\text{C}$  until analysis of drug concentrations (approximately 2 weeks following collection of the final sample).

Urine samples were collected from all horses via free catch when possible or urinary catheter technique via aseptic technique when not (mares only), for measurement of MP concentrations. Samples were collected on Day 0 (prior to drug administration) and on days 1, 2, 3, 4, 6, 7, 10, 13, 14, 17, 21, 24, 28, 35, 42, and 49 post-MPA administration. All samples were tested to ensure that MPA was no longer detected prior to termination of sample collection. All samples were stored at  $-20^{\circ}\text{C}$  until analysis (approximately 2 weeks following administration of the final sample).

#### *Determination of plasma, synovial fluid, and urine methylprednisolone*

The analytical reference standard for MP and the internal standard prednisolone were purchased from Sigma Aldrich (St. Louis, MO, USA). Stock solutions of MP and the internal standard were prepared at 1 mg/mL in methanol. Acetonitrile and water were purchased from Burdick and Jackson (Muskegon, MI, USA), and methyl tert-butyl ether and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The solvents were of HPLC grade or better.

*Plasma sample analysis.* Methylprednisolone working solutions were prepared by dilution of the 1 mg/mL stock solution with methanol to concentrations of 0.01, 0.1, 1, and 10 ng/mL. Plasma calibrators were prepared by dilution of the working standard solutions with drug-free plasma to concentrations of 0.05, 0.1, 0.25, 0.5, 1, 2, 4, 6, 8, and 10 ng/mL. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality control samples (plasma fortified with analyte at four concentrations within the standard curve) were included with each sample set as an additional check of accuracy.

Prior to analysis, 1 mL of plasma was diluted with 100  $\mu\text{L}$  of water containing 0.5 ng/mL of internal standard. The samples were vortexed briefly to mix, and 5 mL of methyl tert-butyl ether was added. The samples were mixed by rotation for 15 min, centrifuged at 2939  $g$  for 5 min, and the top ether layer was removed and dried under nitrogen. Samples were dissolved in 120  $\mu\text{L}$  of 5% acetonitrile in water, both with 0.2% formic acid, and 40  $\mu\text{L}$  was injected into the LC-MS/MS system.

The concentration of MP was measured in plasma by LC-MS/MS using positive electrospray ionization. Quantitative analysis of plasma was performed on a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA, USA) coupled with a turbulent flow chromatography system (TFC TLX4; Thermo Scientific) having 1100 series liquid chromatography systems (Agilent Technologies, Palo Alto, CA, USA). The TLX system was operated in laminar flow mode. Chromatography employed an ACE 3 C18 10 cm  $\times$  2.1 mm, 3  $\mu\text{m}$ , column (Mac-Mod Analytical, Chadds Ford, PA, USA) and a linear gradient of acetonitrile (ACN) in water with a

constant 0.2% formic acid at a flow rate of 0.4 mL/min. The initial ACN concentration was held at 5% for 0.5 min, ramped to 90% over 6.7 min, and held at that concentration for 0.5 min before reequilibrating for 3.5 min at initial conditions.

Detection and quantification were conducted using selective reaction monitoring (SRM) of initial precursor ion for MP (mass-to-charge ratio 375.2 ( $m/z$ )) and the internal standard (361.2  $m/z$ ). The response for the product ions for MP ( $m/z$  185.1, 321.4, 339.2, 357.4) and the internal standard ( $m/z$  147.2, 171.1, 307.3) were plotted and peaks at the proper retention time integrated using Quanbrowser software (Thermo Scientific). Quanbrowser software was used to generate calibration curves and quantitate MP in all samples by linear regression analysis. A weighting factor of  $1/x$  was used for all calibration curves.

The response for MP was linear and gave correlation coefficients ( $R^2$ ) of 0.99 or better. The interday, intraday, analyst-to-analyst precision, and accuracy of the assay were determined by assaying quality control samples in replicates ( $n = 6$ ) for MP. Precision was reported as percent relative standard deviation and accuracy as % nominal concentration. Intraday accuracy was 107% for 0.3 ng/mL, 104% for 1.0 ng/mL, 104% for 4.5 ng/mL, and 89% for 9 ng/mL. Interday accuracy was 101% for 0.3 ng/mL, 98% for 1.0 ng/mL, 97% for 4.5 ng/mL, and 96% for 9 ng/mL. Intraday precision was 9% for 0.3 ng/mL, 8% for 1.0 ng/mL, 7% for 4.5 ng/mL, and 14% for 9 ng/mL. Interday precision was 10% for 0.3 ng/mL, 5% for 1.0 ng/mL, 8% for 4.5 ng/mL, and 9% for 9 ng/mL. The technique was optimized to provide a limit of quantitation of 0.1 ng/mL. The limit of detection (LOD) was approximately 0.05 ng/mL.

*Synovial fluid sample analysis.* Methylprednisolone synovial fluid samples were extracted using the same method as plasma but with an extraction volume of 0.1 mL. Synovial fluid calibrators were prepared by dilution of the working standard solutions with drug-free synovial fluid to concentrations of 1, 2.5, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, 3000, 4000, and 5000 ng/mL. Detection and quantification were the same as plasma. The response for methylprednisolone was quadratic and gave correlation coefficients ( $R^2$ ) of 0.99 or better. Intraday accuracy was 96% for 3 ng/mL, 102% for 750 ng/mL, and 99% for 4000 ng/mL. Interday accuracy was 93% for 3 ng/mL, 102% for 750 ng/mL, and 102% for 4000 ng/mL. Intraday precision was 9% for 3 ng/mL, 3% for 750 ng/mL, and 5% for 4000 ng/mL. Interday precision was 9% for 3 ng/mL, 4% for 750 ng/mL, and 5% for 4000 ng/mL. The technique was optimized to provide a limit of quantitation of 1.0 ng/mL. The LOD was approximately 0.15 ng/mL.

*Urine sample analysis.* Methylprednisolone urine samples were extracted using a modified method of the above plasma and synovial fluid. In brief, 2 mL urine was combined with 100  $\mu\text{L}$  of water containing 5 ng/L of internal standard and 0.8 mL B-glucuronidase enzyme in 1.6 M pH 5 acetate buffer

at 10 000 units/mL (Sigma Aldrich). The pH was adjusted to  $5 \pm 0.5$  using 2N hydrochloric acid and/or 2N sodium hydroxide, and heated in a water bath at 65 °C for 2 h with 99 min of sonication. After cooling to room temperature, 1 mL of 5% ammonium hydroxide in saturated sodium chloride was added and the urine was extracted as above with 5 mL of methyl tert-butyl ether. After drying, the samples were dissolved in 1 mL of pH 6.5 0.6 M phosphate buffer and 2 mL water. The samples were then extracted using C18 solid-phase extraction columns (CUC18 200 MG/3 mL; UCT, Bristol, PA, USA). The columns were conditioned with 2 mL methanol, 3 mL water, samples applied with positive pressure, washed with 3 mL water followed by 2 mL 20% methanol in water, and eluted with 3 mL methanol. The methanol was dried with nitrogen, and the samples were dissolved in 150  $\mu$ L of 5% acetonitrile in water, both with 0.2% formic acid, and 40  $\mu$ L was injected into the LC/MS system. Calibrators were prepared by dilution of the working standard solutions with drug-free urine to concentrations of 0.1, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 200, and 300 ng/mL. Detection and quantification were the same as plasma and synovial fluid; however, only one product ion was used for MP ( $m/z$  185.1). The response for MP was linear and gave correlation coefficients ( $R^2$ ) of 0.99 or better. Intraday accuracy was 102% for 3 ng/mL, 98% for 50 ng/mL, and 95% for 175 ng/mL. Interday accuracy was 97% for 3 ng/mL, 110% for 50 ng/mL, and 94% for 175 ng/mL. Intraday precision was 7% for 3 ng/mL, 4% for 50 ng/mL, and 2% for 175 ng/mL. Interday precision was 11% for 3 ng/mL, 5% for 50 ng/mL, and 3% for 175 ng/mL. The technique was optimized to provide a limit of quantitation of 1.0 ng/mL. The LOD was approximately 0.1 ng/mL.

#### Pharmacokinetic calculations

Nonlinear least square regression was performed on plasma MP concentration vs. time data using commercially available software (Phoenix WinNonlin Version 6.0; Pharsight, Cary, NC, USA). Noncompartmental analysis was used for determination of the terminal rate constant, plasma terminal half-life, and the area under the plasma concentration–time curve (AUC) for MP plasma and synovial fluid concentration data. The AUC was calculated using the log-linear trapezoidal rule and was extrapolated to infinity using the last measured plasma or synovial concentration divided by the terminal slope  $\lambda_z$ . Statistical analyses using commercially available software (SAS, Cary, NC, USA) were used to assess significant differences in plasma and urine concentrations as well as plasma pharmacokinetic parameters between horses in which synovial fluid was collected (group 1) and those in which it was not (group 2). Data were analyzed using a Student's paired t-test based on the differences between the 2 parameters and a nonparametric (Wilcoxon signed rank) test. Significance was set at  $P < 0.05$ . Pharmacokinetic parameters, synovial fluid concentrations, and urine concentrations for MP are reported as mean  $\pm$  SD, median, and range.

## RESULTS

There was not a statistically significant difference in plasma or urine concentrations at any of the time points sampled or in any of the calculated plasma pharmacokinetic parameters between horses from which synovial fluid was collected (group 1) and those from which synovial fluid was not collected (group 2). Individual MP plasma concentration over time curves for individual horses are depicted in Fig. 1. Mean ( $\pm$ SD) MP plasma concentrations are listed in Table 1. MP was below the LOD (0.05 ng/mL) in plasma by 6.25 days (mean) (range 3–10 days) for horses that had synovial fluid removed and 4.81 days (mean) (range 1.5–10 days) for the horses that did not. Pharmacokinetic parameters following MPA administration are listed in Table 2. The mean  $\pm$  SD urine MP concentrations for individual horses are shown in Fig. 2, and the concentrations listed in Table 3. MP was below the LOD (0.1 ng/mL) in urine between 3 and 17 days for horses that had synovial fluid removed and 4 and 14 days for horses that did not. Synovial fluid was collected from 8 of the 16 horses studied, and removal of drug during collection of synovial fluid does not appear to have a significant effect on the plasma or urine elimination or detection time. Mean  $\pm$  SD synovial fluid concentrations of MP following intra-articular administration are listed in Table 4. Albeit at low concentrations, MP was detected in both the left middle and left antebrachio-carpal joints. Right antebrachio-carpal and middle carpal joint synovial fluid concentrations for individual horses are depicted in Fig. 3. MP was below the LOD (0.15 ng/mL) in synovial fluid collected from the right middle and antebrachio-carpal joints between days 14 and 21 and 70 and 77, respectively. Selected pharmacokinetic parameters for MP in synovial fluid of the right antebrachio-carpal joint are listed in Table 5. The AUC,  $\lambda_z$ , and  $t_{1/2}$  for synovial MP con-

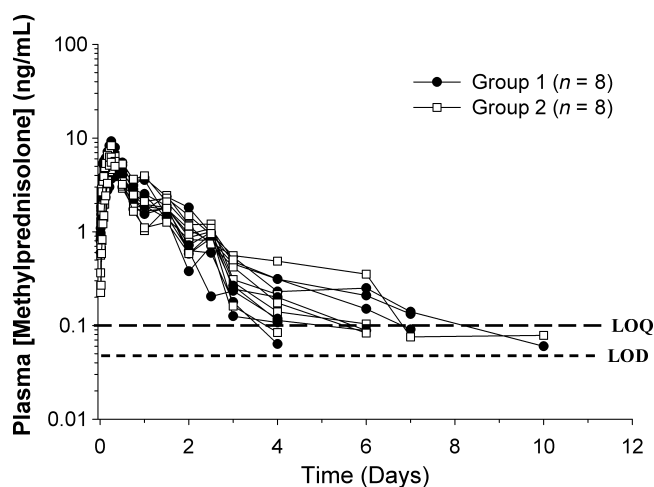


Fig. 1. Individual plasma methylprednisolone concentration over time curves following intra-articular administration of 100 mg of methylprednisolone acetate (Depo-Medrol<sup>®</sup>, Pfizer, New York, NY, USA) into the right antebrachio-carpal joint of 16 exercised Thoroughbred horses. Group 1 and group 2 include horses that had synovial fluid collected and those that did not, respectively.



**Table 1.** Mean ( $\pm$ SD) plasma methylprednisolone concentrations following intra-articular administration of 100 mg of methylprednisolone acetate (Depo-Medrol<sup>®</sup>) in the right antebrachiocarpal joint of 16 exercised Thoroughbred horses

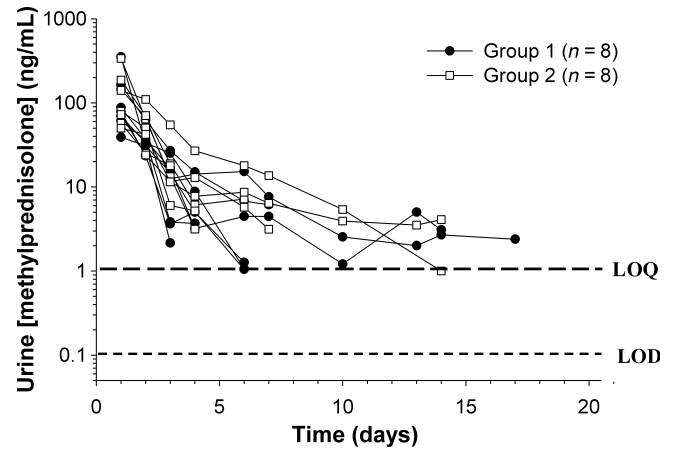
	Plasma [methylprednisolone] (ng/mL)		
	Mean $\pm$ SD		
	Group 1 (n = 8)	Group 2 (n = 8)	Both groups (n = 16)
Baseline	0	0	0
0.25 h	0.805 $\pm$ 0.231	0.800 $\pm$ 0.624	0.803 $\pm$ 0.454
0.5 h	1.39 $\pm$ 0.351	1.58 $\pm$ 1.05	1.48 $\pm$ 0.761
0.75 h	1.89 $\pm$ 0.532	1.99 $\pm$ 1.22	1.94 $\pm$ 0.912
1 h	2.11 $\pm$ 0.566	2.25 $\pm$ 1.28	2.18 $\pm$ 0.960
1.5 h	2.72 $\pm$ 0.667	3.21 $\pm$ 1.63	2.96 $\pm$ 1.23
2 h	3.35 $\pm$ 1.13	3.61 $\pm$ 1.66	3.48 $\pm$ 1.38
2.5 h	3.55 $\pm$ 0.829	4.03 $\pm$ 1.66	3.79 $\pm$ 1.29
3 h	4.08 $\pm$ 1.11	4.15 $\pm$ 1.60	4.11 $\pm$ 1.33
4 h	4.58 $\pm$ 1.28	4.90 $\pm$ 1.75	4.74 $\pm$ 1.49
5 h	5.18 $\pm$ 1.51	5.63 $\pm$ 1.25	5.41 $\pm$ 1.36
6 h	5.82 $\pm$ 1.59	5.92 $\pm$ 1.28	5.87 $\pm$ 1.40
8 h	5.47 $\pm$ 1.38	5.39 $\pm$ 0.741	5.43 $\pm$ 1.07
12 h	4.22 $\pm$ 1.05	3.98 $\pm$ 1.05	4.10 $\pm$ 1.02
18 h	2.74 $\pm$ 1.01	2.41 $\pm$ 0.809	2.57 $\pm$ 0.900
24 h	2.49 $\pm$ 0.982	2.07 $\pm$ 1.13	2.27 $\pm$ 1.04
36 h	1.69 $\pm$ 0.378	1.67 $\pm$ 0.707	1.68 $\pm$ 0.547
48 h	0.791 $\pm$ 0.474	0.978 $\pm$ 0.323	0.878 $\pm$ 0.408
60 h	0.723 $\pm$ 0.287	0.876 $\pm$ 0.330	0.794 $\pm$ 0.307
72 h	0.292 $\pm$ 0.181	0.353 $\pm$ 0.144	0.320 $\pm$ 0.162
96 h	0.166 $\pm$ 0.118	0.203 $\pm$ 0.146	0.172 $\pm$ 0.129
Day 6	0.130 $\pm$ 0.092	0.180 $\pm$ 0.150	0.147 $\pm$ 0.107
Day 7	0.085 $\pm$ 0.053	0.076 $\pm$ 0.0	0.075 $\pm$ 0.049
Day 10	0.060 $\pm$ 0.0	0.078 $\pm$ 0.0	0.069 $\pm$ 0.013
Day 13	ND	ND	
Day 14	ND	ND	
Day 16	ND	ND	
Day 17	ND	ND	
Day 21	ND	ND	
Day 24	ND	ND	
Day 28	ND	ND	
Day 30	ND	ND	

Group 1 – synovial fluid samples collected; Group 2 – synovial fluid samples not collected; Samples collected until Day 44—all ND.

**Table 2.** Pharmacokinetic parameters of methylprednisolone following a single intra-articular (100 mg) administration of methylprednisolone acetate (Depo-Medrol<sup>®</sup>) in the right antebrachiocarpal joint of exercised Thoroughbred horses (n = 16). All values in this table were generated using noncompartmental analysis

	Mean (SD)	Median	Range
$\lambda_z$ (per days)	0.986 $\pm$ 0.812	0.789	0.292–3.35
$t_{1/2\lambda}$ (days)	1.09 $\pm$ 0.666	0.878	0.207–2.38
$T_{max}$ (days)	0.273 $\pm$ 0.855	0.25	0.167–0.33
$C_{max}$ (ng/mL)	6.17 $\pm$ 1.39	5.94	3.88–9.22
$T_{last}$ (days)	5.53 $\pm$ 2.39	6.0	1.5–10.0
$AUC_{last}$ (day ng/mL)	6.35 $\pm$ 1.36	6.33	3.83–8.81
$AUC_{\infty}$ (day ng/mL)	6.52 $\pm$ 1.38	6.47	3.95–9.03

$\lambda_z$ , terminal slope;  $t_{1/2\lambda}$ , terminal half-life;  $T_{max}$ , time to maximal plasma concentration;  $C_{max}$ , maximal plasma concentration; AUC, area under the plasma concentration–time curve.



**Fig. 2.** Individual urine methylprednisolone concentration over time curves following intra-articular administration of 100 mg of methylprednisolone acetate (Depo-Medrol<sup>®</sup>) into the right radiocarpal joint of 16 exercised Thoroughbred horses. Groups 1 and 2 include horses that had synovial fluid collected and those that did not, respectively.

**Table 3.** Mean ( $\pm$ SD) urine methylprednisolone concentrations following intra-articular administration of 100 mg of methylprednisolone acetate (Depo-Medrol<sup>®</sup>) in the right antebrachiocarpal joint of 16 exercised Thoroughbred horses

	Urine [methylprednisolone] (ng/mL)		
	Mean $\pm$ SD		
	Group 1 (n = 8)	Group 2 (n = 8)	Both groups (n = 16)
Baseline	0	0	0
Day 1	125 $\pm$ 101	140 $\pm$ 98.0	111 $\pm$ 86.4
Day 2	40.2 $\pm$ 15.6	48.8 $\pm$ 33.2	35.9 $\pm$ 16.8
Day 3	13.2 $\pm$ 9.62	19.1 $\pm$ 16.3	12.7 $\pm$ 8.12
Day 4	7.88 $\pm$ 4.99	10.3 $\pm$ 8.76	8.10 $\pm$ 4.49
Day 6	4.98 $\pm$ 5.55	9.88 $\pm$ 5.49	5.54 $\pm$ 4.86
Day 7	6.07 $\pm$ 1.58	7.74 $\pm$ 5.39	5.54 $\pm$ 1.76
Day 10	3.77 $\pm$ 1.74	4.67 $\pm$ 1.04	3.83 $\pm$ 1.24
Day 13	1.61 $\pm$ 0.562	3.52 $\pm$ 0.00	2.25 $\pm$ 1.17
Day 14	2.89 $\pm$ 0.281	2.55 $\pm$ 0.0	3.29 $\pm$ 0.722
Day 17	2.39 $\pm$ 0.0	ND	2.39 $\pm$ 0.0
Day 21	ND	ND	ND
Day 24	ND	ND	ND
Day 28	ND	ND	ND
Day 35	ND	ND	ND
Day 42	ND	ND	ND
Day 49	ND	ND	ND

Group 1 – synovial fluid samples collected; Group 2 – synovial fluid samples not collected.

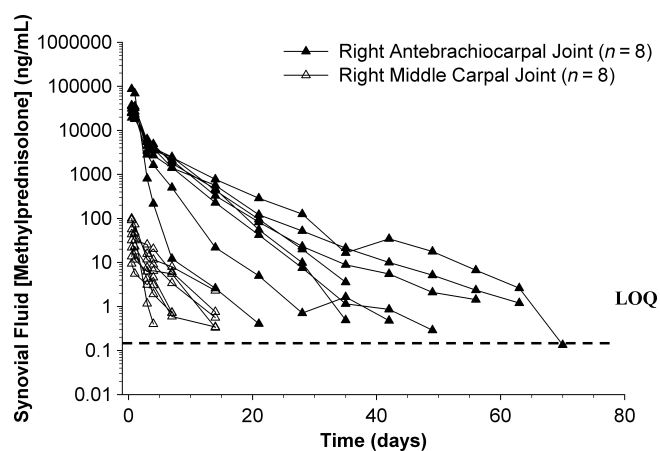
concentrations differed significantly ( $P < 0.05$ ) from those calculated for plasma MP concentrations.

**DISCUSSION**

In the current study, we have described the disposition of MP following right antebrachiocarpal joint intra-articular administration to racing fit Thoroughbred horses. The purpose

**Table 4.** Mean ( $\pm$ SD) synovial fluid methylprednisolone concentrations following a single intra-articular administration of 100 mg of methylprednisolone acetate (Depo-Medrol<sup>®</sup>) in the right antebrachio-carpal joint to exercised Thoroughbred horses ( $n = 8$ )

	[Methylprednisolone] (ng/mL)			
	Right middle carpal joint	Right antebrachio-carpal joint	Left middle carpal joint	Left antebrachio-carpal joint
Baseline	ND	ND	ND	ND
12 h	46.1 $\pm$ 34.4	35 777 $\pm$ 21 481	0.754 $\pm$ 0.438	0.365 $\pm$ 0.268
Day 1	32.1 $\pm$ 22.5	31 819 $\pm$ 16 051	0.541 $\pm$ 0.484	0.193 $\pm$ 0.170
Day 2	–	–	0.157 $\pm$ 0.149	0.145 $\pm$ 0.142
Day 3	9.43 $\pm$ 8.04	4321 $\pm$ 1865	–	–
Day 4	7.16 $\pm$ 6.49	3088 $\pm$ 1512	ND	ND
Day 7	3.56 $\pm$ 3.01	1517 $\pm$ 884	ND	ND
Day 14	0.855 $\pm$ 0.821	352 $\pm$ 264	ND	ND
Day 21	ND	86.2 $\pm$ 91.7	ND	ND
Day 28	ND	34.1 $\pm$ 43.6	ND	ND
Day 35	ND	10.2 $\pm$ 14.0	ND	ND
Day 42	ND	7.63 $\pm$ 8.26	ND	ND
Day 49	ND	6.31 $\pm$ 7.86	ND	ND
Day 56	ND	3.48 $\pm$ 2.79	ND	ND
Day 63	ND	1.90 $\pm$ 0.995	ND	ND
Day 70	ND	0.147 $\pm$ 0	ND	ND
Day 77	ND	ND	ND	ND

**Fig. 3.** Individual synovial fluid methylprednisolone concentration over time curves following intra-articular administration of 100 mg of methylprednisolone acetate (Depo-Medrol<sup>®</sup>) into the right antebrachio-carpal joint of eight exercised Thoroughbred horses.

of this study was twofold. First, we sought to expand upon existing knowledge of the intra-articular pharmacokinetics of MP to provide additional data upon which to establish an appropriate threshold and withdrawal time prior to performance. The second goal was to describe synovial fluid concentrations of MP relative to plasma and urine concentrations following intra-articular administration.

Synovial fluid was only collected from 8 of the 16 horses studied, and therefore, plasma and urine concentrations, as well pharmacokinetic parameters for each group were evaluated separately in order to take into account the affect that removal of drug from the site of administration (the joint) might have on the clearance and elimination of the drug. This is especially important as the ultimate goal of this study is to

extrapolate these results to animals in a clinical, nonresearch setting in which drug is not removed via sampling of synovial fluid. Based on the results of the current study, collection of synovial fluid did not appear to affect the rate of elimination and the length of detection of MP. It is important to note, however, that this study did not assess the implications of removal of MP from the joint on the drug's pharmacodynamic effects.

As part of an ongoing effort to establish threshold values and withdrawal times for therapeutic substances, the Racing Medication and Testing Consortium (RMTC) has proposed a plasma threshold for MP of 0.1 ng/mL for racehorses. In the current study as well as previous studies describing plasma concentrations of MP following intra-articular administration (Autefage *et al.*, 1986; Lillich *et al.*, 1996; Soma *et al.*, 2006; Menendez *et al.*, 2012), relatively low concentrations of drug were detected in plasma at all times sampled. The  $C_{max}$  in the current study is lower ( $6.06 \pm 1.57$  and  $6.27 \pm 1.29$  ng/mL for Groups 1 and 2, respectively) than that reported previously by Soma *et al.* (2006) ( $7.3 \pm 3.3$  ng/mL). However, this difference is to be expected based on the higher dose (200 mg) utilized in the previous study (Soma *et al.*, 2006). The  $C_{max}$  reported by Menendez *et al.* (2012) ( $5.07 \pm 2.97$  ng/mL) following administration of 100 mg of MP (60 and 40 mg in the tarsometatarsal and metatarsophalangeal joints, respectively) is lower than that reported here. Because different joints were treated in the previous study, these results suggest that the selection of the joint to be treated and/or the treatment of multiple joints also affects plasma concentrations. A similar finding was noted with triamcinolone acetonide, whereby a higher  $C_{max}$  was reported when the antebrachio-carpal joint (Knych *et al.*, 2013) was treated as compared with the tarsometatarsal joint (Knych *et al.*, 2011). The apparent lower plasma  $C_{max}$  in all

**Table 5.** Pharmacokinetic parameters describing the disposition kinetics of methylprednisolone in synovial fluid following a single intra-articular (100 mg) administration of methylprednisolone acetate (Depo-Medrol®) in the right antebrachio-carpal joint of exercised Thoroughbred horses ( $n = 8$ ). All values in this table were generated using noncompartmental analysis

	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6	Horse 7	Horse 8	Mean ( $\pm$ SD)	Median
$\lambda_z$ (per days)	0.222	0.234	0.333	0.241	0.100	0.103	0.133	0.228	0.199 $\pm$ 0.081	0.225
$t_{1/2z}$ (days)	3.12	2.96	2.08	2.88	6.94	6.75	5.23	3.05	4.12 $\pm$ 1.90	3.09
AUC <sub><math>\infty</math></sub> (day ng/mL)	67 037	79 535	75 045	131 822	92 633	68 546	89 992	51 635	82 036 $\pm$ 24 025	77 290

$\lambda_z$ , terminal slope;  $t_{1/2z}$ , terminal half-life; AUC, area under the plasma concentration–time curve.

cases is likely attributable to different transfer rates from the different joints to plasma.

When deciding the appropriate time to withdrawal a drug prior to competition, the time until drug concentrations equal or fall below the acceptable threshold value becomes especially important. In the study described here, the average MP plasma concentration remained above the proposed RMTC threshold until 7 days postadministration; however, the individual plasma concentrations for 2 of the 16 horses studied had plasma concentrations above the 0.1 ng/mL threshold (0.13 and 0.14 ng/mL) until the subsequent sample collection (10 days). This is in close agreement with that reported by Soma *et al.* (2006). In that study, investigators reported that two horses had MP plasma concentrations above 0.1 ng/mL (the RMTC proposed threshold), 8 days post-intra-articular administration of 100 mg into the carpal joint. By 10 days postadministration, drug was only detected in one horse, but that concentration was below the proposed threshold (0.60 ng/mL). In a second study,  $T_{\text{last}}$  (based on an LOQ of 0.050 ng/mL) of 7 days was reported following intra-articular administration of 100 mg, split between two joints (60 and 40 mg in the tarsometatarsal and metatarsophalangeal joints). The shorter detection time of MP in plasma suggests that the detection time of MP could be affected by the particular joint treated or by the administration of drug into multiple joints or both. Based on these findings, it is important to note that applying a withdrawal time established for a particular therapeutic regimen to another might not always be appropriate.

In many racing jurisdictions, urine is the matrix used by drug-testing laboratories for regulating the use of drugs in performance horses. MP urine concentrations following intra-articular administration have been described previously (Lillich *et al.*, 1996; Menendez *et al.*, 2012). In the first study to describe urine concentrations, MP or metabolite equivalents were detected for up to 72 h following administration of 100 mg (Lillich *et al.*, 1996). This is a much shorter detection window than reported in the current study. The discrepancy is most likely attributable to differing assay sensitivity. Lillich *et al.* (1996) used a semi-quantitative assay with an LOD of 2.5 ng/mL, whereas in the current study, an LC-MS/MS-based assay with an LOD of 0.1 ng/mL and an LOQ of 1 ng/mL was utilized. Menendez *et al.* (2012) report a detection time of 11 days based on an LOQ of 0.25 ng/mL, which is slightly shorter than that reported in the current study (up to 17 days

based on an LOQ of 0.25 ng/mL). Similar to the plasma results, this may be due to differences in treatment protocols between the two studies (i.e., different joints and/or multiple joints).

Synovial fluid was collected from eight of the sixteen horses studied here, starting at 12 h postdrug administration and continuing until MP was no longer detected. Synovial MP concentrations were much higher and detected for an extended period of time relative to plasma. As discussed previously, MP was not detected in any plasma samples collected after 10 days postadministration. MP was, however, still detected in synovial fluid in the majority of the horses up to 42 days postadministration and for to 70 days in one horse. Autefage *et al.* (1986) reported a detection window ranging from 4.8 to 39 days in synovial fluid following intra-articular administration of 100 mg of MP. The longer detection window in the current study is again likely due to assay sensitivity differences. The relationship between MP synovial concentrations and anti-inflammatory effects is unknown, but the results from this study demonstrate that drug remains at the effector site for a prolonged period of time and for much longer than predicted by plasma concentrations.

Beneficial clinical effects in joints other than the treated one has been noted following intra-articular administration of triamcinolone acetonide (Frisbie *et al.*, 1997). And while synovial drug concentrations were not measured in that study, a subsequent study revealed no detectable triamcinolone acetonide in joints other than the treated one. While to the authors' knowledge, a similar beneficial effect has not been reported for MP, the previous report describing MP concentrations in joints failed to detect drug in any joints other than the treated one (Lillich *et al.*, 1996). Interestingly, and perhaps due to improved analytical sensitivity, in the current study, drug was detected in all joints sampled. While additional pharmacodynamic studies are necessary to define the minimum effective concentration necessary to treat inflammation in the joint, it is nonetheless, interesting to note that drug can reach measurable concentrations in untreated joints. Detection of drug in the right middle carpal joint may be due to direct transfer from the adjacent antebrachio-carpal joint, however, in the case of the joints on the opposite limb, it is reasonable to assume drug most likely travelled via the systemic circulation.

The current study sought to expand upon current knowledge of the pharmacokinetics and disposition of MP in plasma,

urine, and synovial fluid following intra-articular administration of 100 mg into a single joint. Pharmacokinetics and detection times are used to determine appropriate withdrawal times prior to competition, which in turn decreases the incidence of inadvertent positives. Methylprednisolone was detected for up to 13 days in plasma and 21 days in urine and remained above the proposed RMTC plasma threshold for up to 10 days (two horses). It is important to note that a large number of therapeutic protocols for corticosteroid administration are utilized and based on comparisons between different studies, the total dose administered as well as the specific joint and number of joints treated markedly affect the time to reach the threshold concentration. Additionally, in the presently reported study, the synovial concentration remained above the LOD for up to 70 days postadministration in one horse, suggesting that urine and blood concentrations are not indicative of synovial fluid concentrations and likely pharmacologic effect.

#### ACKNOWLEDGMENTS

The authors would like to thank Dr. Eugene Steffey, Michelle Mitchell, Amy Young, Sandy Yim, and Carley Corado for assistance and the Racing Medication and Testing Consortium and the California Horse Racing Board for providing funding for this project.

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