JOURNAL OF
Veterinary Pharmacology and Therapeutics

J. vet. Pharmacol. Therap. **39**, 469–477. doi: 10.1111/jvp.12298.

Pharmacokinetics of methocarbamol and phenylbutazone in exercised Thoroughbred horses

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Knych, H. K., Stanley, S. D., Seminoff, K. N., McKemie, D. S., Kass, P. H. Pharmacokinetics of methocarbamol and phenylbutazone in exercised Thoroughbred horses. *J. vet. Pharmacol. Therap.* **39**, 469–477.

Methocarbamol (MCBL) is commonly used in performance horses for the treatment of skeletal muscle disorders. Current regulatory recommendations for show horses and racehorses are based on a single oral dose of 5 g, although doses in excess of this are often administered. The goal of the current study was to characterize the disposition of MCBL following higher dose administration and administration in combination with another commonly used drug in performance horses, phenylbutazone (PBZ). Exercised Thoroughbred horses were administered various doses of MCBL as a sole agent and MCBL in combination with PBZ. Blood samples were collected at various times, concentrations of MCBL and PBZ measured using LC-MS/MS and pharmacokinetic parameters calculated using compartmental analysis. Following administration of 15 g of MCBL, either as part of a single- or multiple-dose regimen, a number of horses exceeded the Association of Racing Commissioners International and the United States Equestrian Federation's recommended regulatory threshold at the recommended withdrawal time. There was not a significant difference between horses that received only MCBL and those that received MCBL and PBZ. Results of the current study support an extended withdrawal guideline when doses in excess of 5 g are administered.

(Paper received 20 November 2015; accepted for publication 26 January 2016)

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INTRODUCTION

Methocarbamol (MCBL) is a centrally acting skeletal muscle relaxant labeled for use in horses as an 'adjunctive therapy for acute inflammatory and traumatic conditions of the skeletal muscle' as well as to 'reduce muscular spasms'. Based on this indication, MCBL is used extensively in performance and racehorses. The United States Equestrian Federation (USEF) recommends that this drug be administered a minimum of 12 h prior to performance. In horseracing, MCBL, an American Racing Commissioners International (ARCI) Class 4 substance, has a recommended withdrawal time of 48 h. It is important to note that these recommendations are based on oral doses of 5 g/1000lbs for USEF and 5 g/horse for ARCI. The Racing Medication and Testing Consortium (RMTC) also recommends a minimum withdrawal time of 48 h following intravenous administration of 15 mg/kg. The label dose for intravenous administration is 4.4–22 mg/kg and 22–55 mg/kg for moderate and severe conditions, respectively. While there is no FDA-approved label dose for oral administration, two to three times the intravenous dose has been recommended (Cunningham *et al.*, 1992). Additionally, the ARCI recommendation is for a single administration; however, multiple-dose regimens are often times prescribed by veterinary practitioners.

The pharmacokinetics of MCBL have been described previously (Muir *et al.*, 1984, 1992; Cunningham *et al.*, 1992; Rumpler *et al.*, 2014). An elimination half-life of 1.68 (Cunningham *et al.*, 1992) and 2.89 h (Rumpler *et al.*, 2014) has been reported previously for oral administration and 2.96 h for intravenous administration (Rumpler *et al.*, 2014). However, elimination of MCBL is reportedly dose dependent in horses (Muir *et al.*, 1984), and therefore, the potential for a positive regulatory finding in performance horses exists if doses in excess of those recommended by USEF and ARCI are utilized. Further study of clinically used doses and dosing regimens for MCBL is warranted to assess whether a more prolonged withdrawal time recommendation is prudent in the case of higher doses and more frequent administration.

Phenylbutazone (PBZ; Henry Schein Animal Health, Dublin, OH, USA), a nonsteroidal anti-inflammatory drug (NSAID), is another commonly used medication in performance horses. Currently, the use of PBZ is permissible outside of 12 h accord-

ing to the USEF regulations and outside of 24 h in racehorses. It is common practice in both disciplines to administer PBZ and MCBL in close proximity to one another, and positive MCBL findings in some racing jurisdictions have led investigators to speculate that PBZ has the potential to interfere with the clearance of MCBL (Robinson *et al.*, 2014). Based on this hypothesis and the frequent administration of both compounds, further study of potential drug–drug interactions between the two is necessary.

The primary goal of the study described here was to describe the disposition of MCBL using higher therapeutic doses than previously studied as well as multiple-dosing protocols which are reminiscent of dosing regimens used by veterinarians when treating performance horses. Secondarily, we sought to add to existing information regarding potential drug–drug interactions between MCBL and PBZ with respect to clearance by characterizing the *in vivo* pharmacokinetics of both drugs when administered in close proximity.

MATERIALS AND METHODS

Animals

Sixteen university owned exercised adult Thoroughbred horses including eight geldings and eight mares (age: 4–7 years; weight: 419–610 kg) were studied. Prior to and throughout the course of the study (with the exception of days 1 and 5 of the study), horses were exercised 5 days a week. The exercise regimen for these horses consists of 3 days/week on an Equineciser (Centaur Horse Walkers Inc, Mira Loma, CA, USA) (5 min at walk; 30 min trot; 5 min walk) and 2 days/week on a high-speed treadmill (Mustang 2200; Graber AG, Fahrwangen, Switzerland; Day 1: 5 min @1.6 m/s; 5 min @ 4 m/s; 5 min @ 7 m/s; 5 min @ 1.6 m/s all at 6% incline. Day 2: 3 minute @ 1.6 m/s; 4 min @ 4.0 m/s; 2 min @ 7.0 m/s; 2 min @ 11.0 m/s and 5 min @1.6 m/s all at 3% incline).

Before beginning the study, horses were determined healthy and free of disease 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. Food was withheld for 1 h following drug administration, and water was available *ad libitum* throughout the study. Horses did not receive any other medications for at least 4 weeks prior to commencement of this study. This study was approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

Instrumentation and drug administration

The dosing regimens were selected based on input from equine practitioners (Table 1). Horses were randomly assigned to each dose group. Randomization was repeated 6 times until all dose groups had a total of eight horses. The tablet formulation of MCBL (Robaxin; Camber Pharmaceuticals, Piscataway Township, NJ, USA) was dissolved in 45–60 mL of water in a dosing syringe and administered directly into the oral cavity. The paste formulation of MCBL was also administered directly into the oral cavity. The intravenous MCBL formulation was administered directly into the jugular vein via a needle and syringe. As there is no commercially available paste formulation and the FDA-approved injectable formulation is currently unavailable, both formulations were compounded for the purposes of this study. Both formulations were tested for purity and potency prior to administration (see drug concentration determination). Commercially available, FDA-approved PBZ formulations were used for both oral (ButaPaste; Henry Schein Animal Health) and intravenous (Aspen Veterinary Resources LTD, Liberty, MO, USA) administration. When administered together, MCBL was administered first, followed by PBZ. A minimum washout period of 2 weeks was allowed to elapse before subsequent dosing. Prior to drug administration on the first and last day, a 14-gauge intravenous catheter was percutaneously placed in one external jugular vein of all horses for sample collection. Each horse was weighed preceding the initial drug administration.

Sample collection

Blood samples were collected at time 0 (prior to drug administration) and at 5, 10 (intravenous administration only), 15, 30, and 45 min, and 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 18, 24, 30 and 36, 48, and 72 h postdrug administration following the first dose and last dose (Day 5 for MCBL groups only and days 5 and 6 for MCBL + PBZ groups). Catheters were removed following collection of the 18-hour sample, and the remaining samples were collected by direct venipuncture. Samples collected during the dosing period were collected immediately prior to drug administration. Blood samples were collected into serum separator tubes (Kendall/Tyco Healthcare, Mansfield MA, USA) and were centrifuged at 3000 × **g** for 10 min. Serum was immediately transferred into storage cryovials (Phenix Research Products; Chandler, NC, USA) and stored at −20 °C until analyzed.

Urine was collected by free catch at 0, 24, 48, and 72 h postdrug administration. All samples were collected within 15 min of the actual time point. Urine samples were stored at −20 °C until analyzed.

Analytical method and validation

Serum sample analysis. For analysis, MCBL and PBZ were combined into one working solution. The working solution was prepared by dilution of a 1 mg/mL stock solutions with methanol to concentrations of 0.01, 0.1, 1, 10, and 100 ng/μL. Serum calibrators were prepared by dilution of the working standard solutions with drug-free equine serum to concentrations ranging from 1–60 000 ng/mL. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality control samples

Table 1. Methocarbamol (MCBL) and phenylbutazone (PBZ) dosing regimens. Each group included 8 exercised Thoroughbred horses

Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Group 1	MCBL IV 2 g	–	–	–	–	–
Group 2	MCBL tablets 5 g SID	–	–	–	–	–
Group 3	MCBL paste 5 g SID	–	–	–	–	–
Group 4	MCBL tablets 15 g SID	–	–	–	–	–
Group 5	MCBL paste 15 g SID	–	–	–	–	–
Group 6	MCBL tablets 15 g BID	MCBL tablets 15 g BID	MCBL tablets 15 g BID	MCBL tablets 15 g BID	MCBL IV 2 g SID	–
Group 7	MCBL paste 15 g BID	MCBL paste 15 g BID	MCBL paste 15 g BID	MCBL paste 15 g BID	MCBL IV 2 g SID	–
Group 8	MCBL tablets 15 g BID	MCBL tablets 15 g BID	MCBL tablets 15 g BID	MCBL tablets 15 g BID	MCBL IV 2 g SID	PBZ IV 2 g SID
Group 9	MCBL paste 15 g BID	MCBL paste 15 g BID	MCBL paste 15 g BID	MCBL paste 15 g BID	MCBL IV 2 g SID	PBZ IV 2 g SID
Group 10	PBZ paste 2 g SID	PBZ paste 2 g SID	PBZ paste 2 g SID	PBZ paste 2 g SID	PBZ paste 2 g SID + MCBL IV 2 g SID	PBZ IV 2 g SID
Group 11	PBZ paste 2 g SID	PBZ paste 2 g SID	PBZ paste 2 g SID	PBZ paste 2 g SID	PBZ paste 2 g SID + MCBL tablets 15 g SID	PBZ IV 2 g SID
Group 12	PBZ paste 2 g SID	PBZ paste 2 g SID	PBZ paste 2 g SID	PBZ paste 2 g SID	PBZ paste 2 g SID + MCBL paste 15 g SID	PBZ IV 2 g SID

(serum fortified with analyte at four (for MCBL) and three (for PBZ) concentrations within the standard curve) were included with each sample set as an additional check of accuracy.

Prior to analysis, 500 μL of serum was diluted with 500 μL of acetonitrile (ACN):1M acetic acid (9:1, v:v) containing 200 ng/mL of d4-MCBL and d10-PBZ internal standards, to precipitate proteins. The samples were vortexed on a Glas-Col Large Capacity Mixer (Glass-Col, Terre Haute, IN, USA) for 2 min to mix, refrigerated for 20 min, vortexed for an additional 1.5 min, and centrifuged at 4300 rpm/3830 **g** for 10 min at 4 °C and 30 μL injected into the LC/MS system.

The concentration of MCBL and PBZ was measured in serum by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Quantitative analysis of serum 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) with an 1100 series liquid chromatography system (Agilent Technologies, Palo Alto, CA, USA) and operated in laminar flow mode. The system was operated using positive electrospray ionization (ESI(+)). The spray voltage was set at 3500 V; sheath gas and auxiliary gas were 50 and 20, respectively (arbitrary units); vaporizer temperature was 0; and capillary temperature was 270 °C. Product masses and collision energies were optimized by infusing the standards into the mass spectrometer. Chromatography employed an ACE 3 C18 10 cm × 2.1 mm 3 μm column (Mac-Mod Analytical, Chadds Ford, PA, USA) and a linear gradient of ACN in water, both with 0.2% formic acid, at a flow rate of 0.40 mL/min. The initial ACN concentration was held at 1% for 0.5 min, ramped to 60% over 5 min and ramped to 95% over 1 minute, and held at that concentration for 0.33 minute, before re-equilibrating for 3.83 min.

Detection and quantification were conducted using selective reaction monitoring (SRM) of initial precursor ion for MCBL (mass-to-charge ratio *(m/z)* 242.105), PBZ (*(m/z)* 309.157) and the internal standards d4-MCBL (*(m/z)* 246.126) and d10-PBZ (*(m/z)* 319.221). The response for the product ions for MCBL (*m/z* 121, 199.2), PBZ (*m/z* 92.1) and the internal standards d4-MCBL (*m/z* 125.1, 203.1) and d10-PBZ (*m/z* 221.1) 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 analytes in all samples by linear regression analysis. A weighting factor of 1/X was used for all calibration curves.

The response for MCBL and PBZ was linear and gave correlation coefficients (R^2) of 0.99 or better. Accuracy was reported as percent nominal concentration, and precision was reported as percent relative standard deviation (Table 2). The technique was optimized to provide a limit of quantitation (LOQ) of 1 ng/mL and a limit of detection (LOD) of approximately 0.7 ng/mL for MCBL and a LOQ of 10 ng/mL and LOD detection of approximately 5 ng/mL for PBZ.

Urine sample analysis. Working solutions were prepared the same as described for serum analysis. Urine calibrators were prepared by dilution of the working standard solutions with drug-free equine urine to concentrations of 10–13 000 ng/mL. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality control samples (equine urine fortified with analyte at two concentrations within the standard curve) were included with each sample set as an additional check of accuracy.

Prior to analysis, 1 mL of urine was diluted with 0.1 mL of water containing 2000 ng/mL of d4-MCBL and d10-PBZ internal standards and 0.4 mL of β-glucuronidase enzyme, (Sigma-Aldrich, St. Louis, MO, USA) at 10 000 Units/mL in pH 5, 1.6 M acetate buffer. The pH of the samples was adjusted to 5 ± 0.5 with 2 N NaOH or 2 N HCl, as necessary, and heated in a sonicating water bath at 65 °C for 2 h with 99 min of sonication. After cooling to room temperature, the pH was adjusted to 6 ± 0.5 with 1.5 mL of pH 6.5, 0.6 M phosphate buffer and 2 N NaOH or 2 N HCl, as necessary. Samples were mixed gently and centrifuged at 4000 rpm/3310 **g** for 5 min at 4 °C. The samples were subjected to solid-phase extraction using CleanScreen Extraction Columns DAU (130 mg/3 mL) (United Chemical Technologies, Bristol, PA, USA). In brief, the columns were conditioned with 2 mL of methanol, and then 3 mL of 0.1M phosphate buffer at pH 6 before the samples was loaded onto the column. The samples were passed through the column using a CEREX system 48 Processor with positive pressure SPE manifold (SPE Ware; Baldwin Park, CA, USA) for a minimum of 2 min. The columns were rinsed consecutively with 3 mL of water, 2 mL of 1M acetic acid prior to elution with 3 mL (1:1, v:v) hexane:ethyl acetate. Samples were dried under nitrogen in a Zymark TurboVap (McKinley Scientific, Sparta, NJ, USA) at 45 °C, reconstituted in 150 μL of 5% ACN in water, both with 0.2% formic acid and 30 μL injected into the LC/MS system. Detection and quantification were the same as described above.

The response for methocarbamol and PBZ was linear and gave correlation coefficients (R^2) of 0.99 or better. Accuracy was reported as percent nominal concentration and precision was reported as percent relative standard deviation (Table 2). The technique was optimized to provide a LOQ of 10 ng/mL and a LOD of approximately 5 ng/mL for MCBL and an LOQ of 25 ng/mL and a LOD of approximately 10 ng/mL for PBZ.

Methocarbamol dosing solution analysis. The MCBL injectable solution (label concentration of 100 mg/mL) was serially diluted with water to concentrations of 4000 and 4 μg/mL in duplicate. The MCBL oral paste (label concentration of 500 mg/mL) was serially diluted with water/ACN mixture to concentrations of 50 000, 500, and 5 μg/mL prepared in quadruplicate. The calibration curves described above for serum analysis were used for quantitation. Ten microliters of the each dosing solution was injected into the LC/MS system and samples quantitated as described above.

Pharmacokinetic calculations

Pharmacokinetic analysis was performed on serum MCBL and PBZ concentrations using compartmental analysis and a commercially available software program (Phoenix WinNonlin version 6.2; Pharsight, Cary, NC, USA). Serum concentration data were modeled using either a library or a user-defined 2-compartment model to account for multiple doses and multiple routes of administration. For groups receiving oral doses followed by an intravenous dose, serum concentrations were modeled simultaneously. Data points (serum concentrations)

Table 2. Accuracy and precision values for LC-MS/MS analysis of methocarbamol and phenylbutazone in equine serum and urine

Drug	Matrix	Concentration (ng/mL)	Intraday accuracy (% nominal concentration)	Intraday precision (% relative SD)	Interday accuracy (% nominal concentration)	Interday precision (% relative SD)
Methocarbamol	Serum					
		3.0	102	10.0	101	14.0
		40.0	106	5.0	105	4.0
		200.0	101	5.0	101	3.0
		40 000	92.0	4.0	96.0	3.0
	Urine					
		750.0	108	3.0	108	4.0
		9000	106	2.0	106	3.0
Phenylbutazone	Serum					
		40.0	106	5.0	106	5.0
		200.0	94.0	4.0	90.0	4.0
		40 000	109	3.0	108	3.0
	Urine					
		750.0	112	4.0	110	3.0
		9000	108	3.0	105	4.0

included in analysis were serum concentration values until drug concentrations fell between the LOQ and LOD (Jusko, 2012). Coefficient of variation, Akaike information criterion (Yamaoka *et al.*, 1978), and visual inspection of the residual plots were used to determine the goodness of fit of the model and the appropriate weighting scheme for the individual horse data. The AUC_{last} was calculated using the log-linear trapezoidal method. Pharmacokinetic parameters and serum and urine concentrations for MCBL and PBZ are reported as mean ± SD and median (range).

Statistical analysis

Due to the large number of dosing groups included in this study, only pharmacokinetic parameters from selected groups were compared statistically. Statistical comparisons for horses receiving MCBL included groups 1 to 10, groups 2 to 3, groups 4 to 2, groups 4 to 5, groups 5 to 3, groups 6 to 8, groups 7 to 9, groups 6 to 7, groups 11 to 12, groups 11 to 4 and groups 12 to 5. Statistical comparisons for horses receiving PBZ included groups 8 to 9 and groups 11 to 12.

Mixed-effects analysis of variance was used to account for the inclusion of individuals in multiple groups. Prior-specified *post hoc* comparisons between groups were made using either two-group Student's *t*-tests when no horses were in both of the groups, or mixed-effects ANOVAs for two groups when some horses were present in both groups selected for *post hoc* testing. A Bonferroni–Holm multiple-comparison adjustment for *post hoc* tests was used to preserve a nominal Type I error of 0.05.

RESULTS

The concentration of the injectable and paste MCBL formulations was 94.3 mg/mL (94.3% of the labeled dose) and 484 mg/mL (97.0% of the labeled dose), respectively. Mean

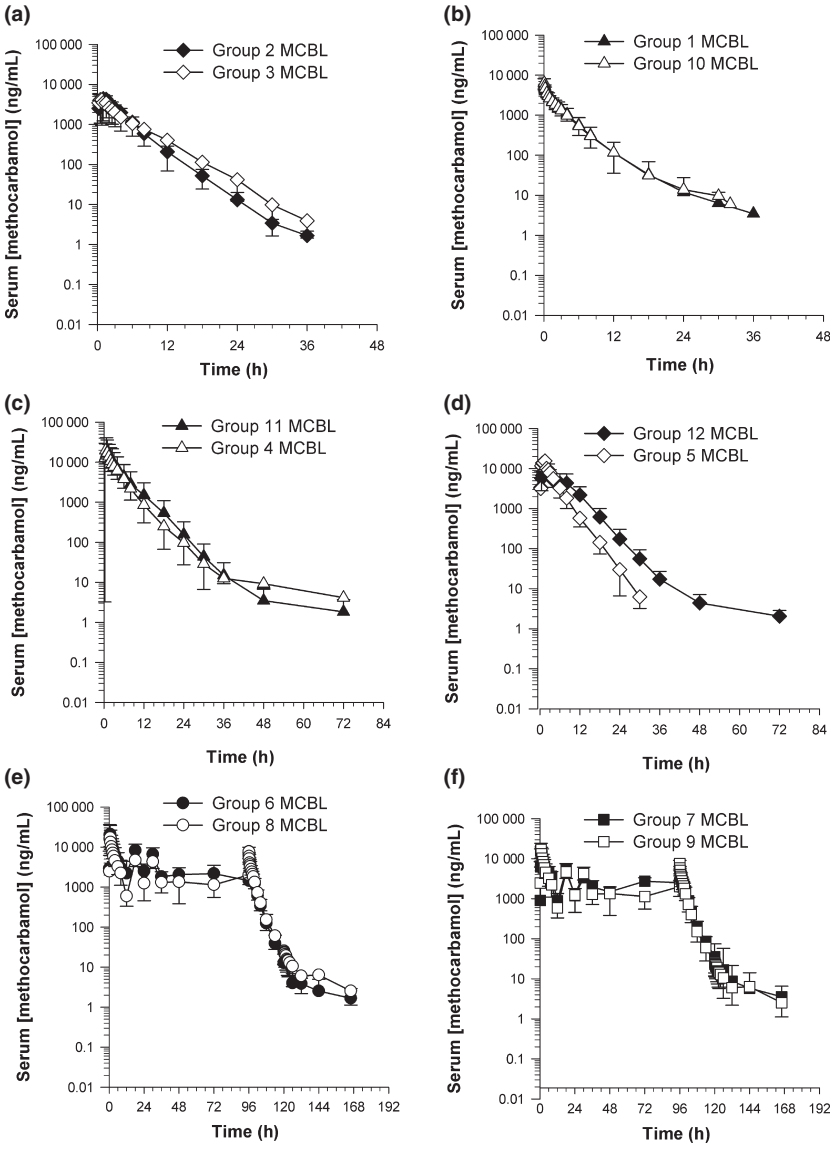

Fig. 1. Plasma concentration–time curve for methocarbamol following single intravenous administration (Group 1; 2 g) and oral administrations (groups 2 and 3; 5 g) (groups 4 and 5; 15 g) or multiple oral administrations (15 g BID × 4.5 days) (groups 11 and 12) or multiple oral administrations (15 g BID × 4 days) followed by a single intravenous administration (2 g) (groups 6, 7, 8, 9, and 10) either with (Groups 6, 7, 8, 9, 10, 11, and 12) or without (groups 1, 2, 3, 4, and 5) phenylbutazone to Thoroughbred horses.

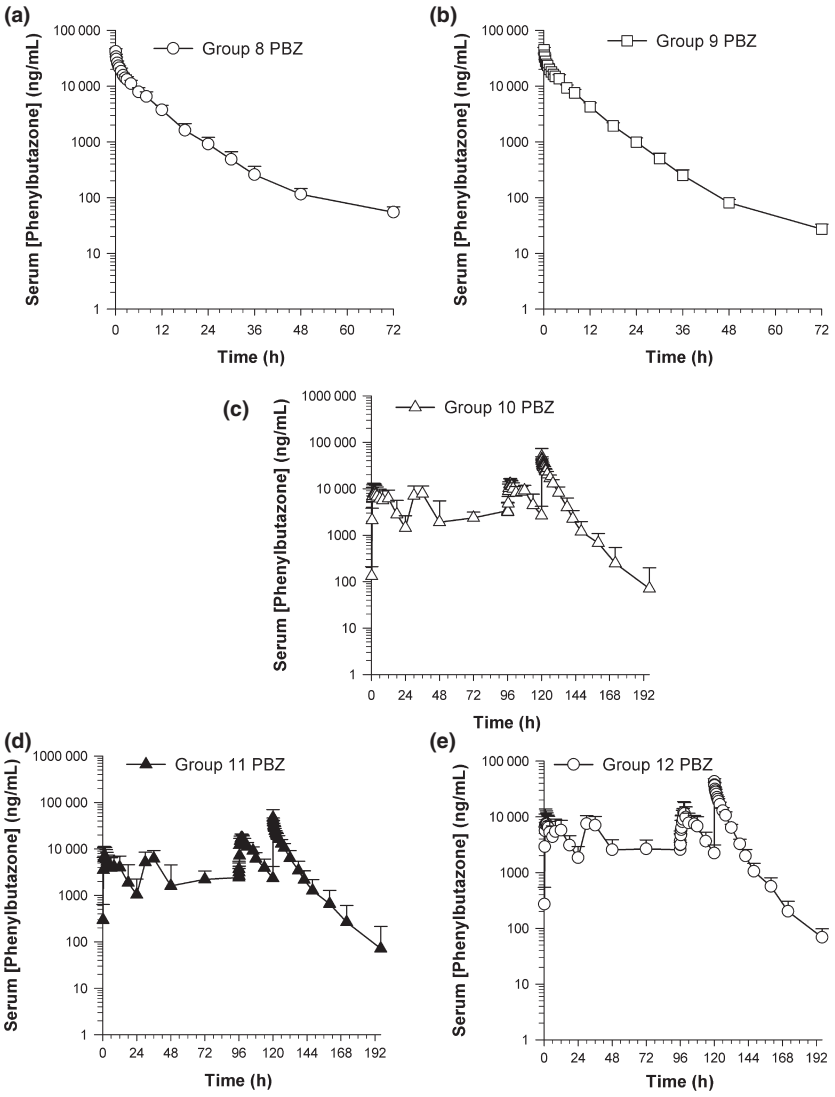

Fig. 2. Plasma concentration versus time curve for phenylbutazone following multiple oral administrations (2 g SID × 5 days) followed by a single intravenous administration (2 g) (groups 10, 11, and 12) or a single intravenous administration (2 g) (groups 8 and 9) in combination with methocarbamol to Thoroughbred horses (n = 8/group).

Table 3. Pharmacokinetic parameters for methocarbamol (mean ± SD (median)) following administration of various dosing regimens to exercised Thoroughbred horses (n = 8/group)

Parameters	$T_{1/2\alpha}$ (h)‡	$T_{1/2\beta}$ (h)‡	AUC_{last} (h·μg/mL)	Vd_{ss} (L/kg)	Cl_B(mL/kg/min)
Group 1	0.181 ± 0.762 (0.818)	2.90 ± 1.03 (2.76)	12.6 ± 3.53 (12.1)	1.14 ± 0.151 (1.09)	5.90 ± 1.10 (5.93)
Group 2	0.072 ± 0.901 (0.857)	3.11 ± 0.579 (3.04)	19.1 ± 3.19[d] (18.5)	–	–
Group 3	0.279 ± 0.533 (0.400)	3.37 ± 0.827 (3.37)	19.4 ± 9.95[e] (16.4)	–	–
Group 4	1.48 ± 0.897 (1.74)	4.83 ± 6.10 (4.13)	72.2 ± 28.1[b] (65.9)	–	–
Group 5	0.376 ± 0.298 (0.782)	2.64 ± 0.487 (2.71)	54.8 ± 18.8[c] (50.4)	–	–
Group 6	0.606 ± 0.304 (0.672)	4.14 ± 0.930 (4.21)	84.0 ± 32.3 (78.7)	2.13 ± 1.00 (1.94)	7.03 ± 2.16 (6.44)
Group 7	0.730 ± 0.363[i] (0.750)	4.28 ± 2.21 (4.84)	81.8 ± 32.2 (62.7)	1.78 ± 0.428 (1.77)	6.15 ± 1.80 (6.86)
Group 8	0.538 ± 0.140 (0.547)	4.70 ± 0.129 (5.01)	72.6 ± 21.9 (65.8)	2.17 ± 0.629 (2.06)	6.64 ± 1.53 (6.78)
Group 9	0.600 ± 0.409[gj] (0.716)	3.34 ± 1.86 (3.78)	84.3 ± 22.1 (83.6)	1.36 ± 1.30 (0.371)	6.41 ± 1.15 (6.45)
Group 10	0.206 ± 0.431[i] (0.23)	2.67 ± 0.396 (2.55)	12.5 ± 3.48 (10.9)	1.13 ± 0.133 (1.13)	5.95 ± 1.16 (6.23)
Group 11	0.152 ± 0.170 (0.170)	6.04 ± 3.05 (6.92)	81.0 ± 25.8 (73.3)	–	–
Group 12	0.833 ± 1.05 (0.505)	4.36 ± 2.59 (3.89)	70.1 ± 18.5 (23.1)	–	–

‡harmonic mean. Significant differences ($P < 0.05$): [a]from Group 1, [b]from Group 2, [c]from Group 3, [d]from Group 4, [e]from Group 5, [f]from Group 6, [g]from Group 7, [h]from Group 8, [i]from Group 9, [j]from Group 10, [k]from Group 11, [l]from Group 12.

$T_{1/2\alpha}$, alpha half-life; $T_{1/2\beta}$, beta half-life; AUC_{last}, area under the curve until the last time point; Vd_{ss}, volume of distribution at steady-state; Cl_B, total systemic clearance.

MCBL and PBZ serum concentration versus time curves are depicted in Figs 1 and 2, respectively. Selected pharmacokinetic parameters for MCBL and PBZ are listed in Tables 3 and 4, respectively. With the exception of the AUC_{last}, there were no significant differences in pharmacokinetic parameters between horses that were administered a single 5 g oral dose of MCBL and those that received a single 15 g oral dose. There was no significant difference in the clearance or elimination half-life ($T_{1/2\beta}$) between any of the groups receiving MCBL only and those receiving MCBL and PBZ regardless of whether multiple doses of MCBL or PBZ were administered. Mean (± SD) and median serum MCBL concentrations at 12, 24, 48, and 72 h are listed in Table 5. Following a single 2-gram intravenous administration (Group 1) or a single oral dose of 5 g (Group 2) of MCBL tablets, serum concentrations were below the USEF (0.5 μg/mL) and RMTC (1 ng/mL)-recommended regulatory threshold by 12 and 48 h, respectively. Serum MCBL concentrations exceeded the USEF regulatory threshold at 12 h in 3/8 horses receiving 5 g of MCBL compounded paste (Group 3). Concentrations of MCBL were below the USEF regulatory threshold by 12 h in all groups receiving the final MCBL dose as an intravenous administration (groups 6, 7, 8, 9, and 10). MCBL concentrations remained above the USEF regulatory threshold in 5/8 horses in groups 4 and 5, all horses in groups 11 and 7/8 horses in Group 12 at 12 h postadministration of the final dose. Concentrations were below the USEF regulatory threshold in all horses by 24 h in groups 4, 5, and 12 and by 48 h in Group 11. MCBL levels remained above the ARCI-recommended regulatory threshold at 48 h in 2/8 horses in Group 4, 0/8 horses in Group 5, 7/8 horses in Group 6, 8/8 horses in Group 7, 7/8 horses in Group 8, 4/8 horses in Group 9, 0/8 horses in Group 10, 7/8 horses in Group 11, and 7/8 horses in Group 12. At 72 h postadministration of the final dose, MCBL concentrations were still above the ARCI threshold in 1/8 horses in Group 4, 4/8 horses in Group 6, 7/8 horses in Group 7, 3/8 horses in Group 8, 4/8 horses in Group 9, 2/8 horses in Group 11, and 3/8 horses in Group 12. Mean (± SD) urine concentrations are listed in Tables 6 and 7 for MCBL and PBZ, respectively.

DISCUSSION

MCBL is commonly administered to performance horses and dosing regimens vary between practitioners. As the current regulatory recommendation for oral MCBL in performance horses is based on a single low dose (5 g) administration, the goal of the current study was to describe the disposition of

Table 4. Pharmacokinetic parameters for phenylbutazone (mean ± SD (median)) following administration of various dosing regimens to exercised Thoroughbred horses (n = 8/group)

Parameters	$T_{1/2\alpha}$ (h)‡	$T_{1/2\beta}$ (h)‡	AUC_{last} (h·μg/mL)	Vd_{ss} (L/kg)	Cl_B(mL/kg/min)
Group 8	1.44 ± 1.28 (2.48)	8.81 ± 4.40 (8.50)	160.7 ± 24.2 (159.8)	0.181 ± 0.016 (0.184)	0.382 ± 0.046 (0.370)
Group 9	1.40 ± 1.38 (3.64)	9.39 ± 2.96 (10.5)	189.3 ± 25.4 (190.8)	0.163 ± 0.017 (0.160)	0.368 ± 0.029 (0.364)
Group 10	1.03 ± 1.42 (2.60)	7.22 ± 3.49 (10.3)	184.7 ± 21.6 (192.1)	0.180 ± 0.021 (0.177)	0.384 ± 0.087 (0.358)
Group 11	1.79 ± 1.05 (2.29)	10.1 ± 3.01 (10.7)	170.1 ± 30.9 (162.1)	0.202 ± 0.026 (0.200)	0.365 ± 0.066 (0.359)
Group 12	0.858 ± 1.48 (2.13)	9.57 ± 3.95 (10.7)	159.1 ± 28.1 (163.0)	0.205 ± 0.036 (0.214)	0.392 ± 0.083 (0.385)

‡harmonic mean.

$T_{1/2\alpha}$, alpha half-life; $T_{1/2\beta}$, beta half-life; AUC_{last}, area under the curve until the last time point; Vd_{ss}, volume of distribution at steady-state; Cl_B, total systemic clearance.

Table 5. Selected methocarbamol serum concentrations following administration of various dosing regimens to exercised Thoroughbred horses (n = 8/group)

	MCBL serum concentrations (mean ± SD (median))			
	12 h	24 h	48 h	72 h
Group 1	115.2 ± 97.3 (77.0)	12.0 ± 15.8 (6.0)	ND	ND
Group 2	206.7 ± 76.6 (198.7)	13.1 ± 6.95 (12.4)	ND	ND
Group 3	402.5 ± 333.8 (272.6)	41.5 ± 29.5 (40.8)	ND	ND
Group 4	**855.3 ± 547.0 (746)**	97.2 ± 69.7 (87.0)	**9.26 ± 2.57 (9.0)**	4.00 ± 0 (4.00)
Group 5	**576.5 ± 225.8 (638)**	30.0 ± 23.4 (23.0)	ND	ND
Group 6	124.9 ± 81.7 (98.2)	12.8 ± 8.37 (10.1)	**2.53 ± 2.47 (1.30)**	1.67 ± 1.24 (1.20)
Group 7	197.3 ± 97.0 (184.0)	35.5 ± 31.7 (31.0)	**5.84 ± 3.80 (4.9)**	3.57 ± 2.87 (2.3)
Group 8	151.8 ± 68.8	25.1 ± 13.2 (23.0)	**6.38 ± 6.48 (4.16)**	2.54 ± 1.41 (2.00)
Group 9	130.9 ± 74.1 (104.0)	34.0 ± 39.6 (18.0)	**7.38 ± 8.44 (3.37)**	3.78 ± 2.98 (3.77)
Group 10	116.2 ± 80.5 (87.0)	13.8 ± 18.4 (3.45)	ND	ND
Group 11	**1505.2 ± 1164.9 (1182)**	157.0 ± 173.8	**3.50 ± 2.29 (3.26)**	1.83 ± 0.814 (2.20)
Group 12	**2193.8 ± 1316.6 (2130)**	174.3 ± 131.5 (156)	**4.40 ± 2.76 (4.00)**	2.05 ± 0.819 (2.10)

Bold text indicates that at least one horse in the group exceeded the USEF (500 ng/mL;12 h) or the ARCI (1 ng/mL; 48 h) threshold.

Table 6. Methocarbamol urine concentrations (mean ± SD) following administration of various dosing regimens to exercised Thoroughbred horses (n = 8/group)

	Methocarbamol urine concentrations (ng/mL)		
	24 h	48 h	72 h
Group 1	1232.4 ± 690.3	48.1 ± 24.3	10.5 ± 5.2
Group 2	1782.5 ± 825.2	37.9 ± 28.9	11.6 ± 11.0
Group 3	2905.4 ± 1745.1	92.1 ± 38.2	20.6 ± 11.2
Group 4	7599.1 ± 4127.1	349.8 ± 210.6	63.0 ± 39.0
Group 5	4331.4 ± 2134.4	209.3 ± 181.3	29.1 ± 20.7
Group 6	2297.9 ± 1730.0	329.8 ± 261.9	322.6 ± 344.3
Group 7	5502.4 ± 2907.4	1024.1 ± 1841.6	817.3 ± 414.8
Group 8	2595.2 ± 1839.1	1177.9 ± 1831.8	372.8 ± 96.8
Group 9	1741.7 ± 826.2	307.9 ± 265.9	295.4 ± 259.8
Group 10	1093.0 ± 900.1	53.4 ± 53.1	17.6 ± 6.9
Group 11	13 510.1 ± 14 767.5	552.4 ± 343.7	189.1 ± 211.4
Group 12	27 375.8 ± 26 930.0	701.9 ± 455.1	201.8 ± 146.9

Table 7. Phenylbutazone urine concentrations (mean ± SD) following administration of various dosing regimens to exercised Thoroughbred horses (n = 8/group)

	Phenylbutazone urine concentrations (ng/mL)		
	24 h	48 h	72 h
Group 8	2792.2 ± 1481.2	197.4 ± 106.9	58.4 ± 21.7
Group 9	4282.3 ± 2723.5	310.9 ± 200.8	86.7 ± 31.3
Group 10	10 576.0 ± 5110.3	870.3 ± 381.7	257.2 ± 117.8
Group 11	3270.7 ± 1351.4	369.5 ± 128.3	138.8 ± 66.9
Group 12	2857.1 ± 1262.3	368.4 ± 143.1	104.4 ± 26.9

MCBL following administration of a higher dose than that described previously as well as following multiple administrations. In addition, we sought to characterize the disposition of MCBL when administered with another commonly used medication in performance horses, PBZ.

The systemic clearance and elimination half-life following a single 2 g intravenous dose was in close agreement with that previously reported for a single intravenous dose of 15 mg/kg (Rumpler *et al.*, 2014). Similarly, the elimination half-life following a single 5 g oral dose in the current study was in agreement with that reported previously for a single oral dose of 5 g (Cunningham *et al.*, 1992; Rumpler *et al.*, 2014). Following administration of a single intravenous dose of 2 g or a single oral dose of 5 g, serum concentrations fell below both the USEF- and RMTC-recommended regulatory threshold by the recommended withdrawal times.

The detection time and time above the ARCI-recommended regulatory threshold following administration of both single and multiple doses of 15 g of MCBL were more prolonged in the current study compared with the previous reports that utilized a lower dose (Rumpler *et al.*, 2014). Fifteen of sixteen horses exceeded the ARCI threshold recommendation at 48 h (the current recommended withdrawal time guideline) postadministration of the final dose. It has been suggested previously that the clearance of MCBL is dose dependent with decreased rates of clearance and a longer elimination half-life reported as intravenous doses increased from 4.4–17.6 mg/kg (Muir *et al.*, 1984). The elimination half-life for the 15 g dose was within the range of that reported previously (Rumpler *et al.*, 2014) and was not significantly different from that determined for horses that received a single 5 g dose in the current study. Furthermore, the elimination half-life was not significantly different between horses that received a single 15 g dose and those administered multiple 15 g oral doses followed by a single IV administration, suggesting that elimination is still first order following oral administration of doses ranging from 5–15 g. These findings along with a similar absorption half-life between the groups indicate that the difference in the time above the recommended regulatory threshold is simply due to higher drug exposure as a result of higher dose administration.

An increase in positive MCBL regulatory findings in some racing jurisdictions has led to the suggestion that combination treatment with PBZ may prolong the elimination of MCBL (Robinson *et al.*, 2014). Robinson and colleagues (2014) postulated that this was because the clearance of MCBL was significantly decreased when administered following multiple doses of PBZ. In the current study, we tested this theory by administering multiple doses of PBZ followed by MCBL. In contrast to reports by Robinson and colleagues (2014), in the current study, there was not a significant difference in the elimination of MCBL or the time at which concentrations fell below the regulatory thresholds between the groups that received PBZ and those that did not. It should be noted that in the current study, that a higher dose of MCBL was utilized and that the final dose of PBZ was administered 24 h following administration of MCBL, whereas in the Robinson *et al.* study (2014), a dose of 5 g of MCBL was given and the final dose of PBZ was administered concurrently with MCBL. Timing of PBZ administration in relation to MCBL in the current study was based on the regulatory withdrawal time recommendation of 24 h for PBZ and 48 h for MCBL. In an effort to fully characterize the *in vivo* effect of combination MCBL/PBZ treatment, the dosing regimen was also reversed with MCBL being administered orally for 4 days (BID) followed by a single IV administration at 48 h and PBZ at 24 h. Similar to the first dosing regimen, the clearance of MCBL was not significantly altered.

Arguably, the most common drug–drug interaction affecting clearance is competition for metabolic enzymes. With respect to MCBL and PBZ, both compounds are extensively metabolized prior to elimination (Bruce *et al.*, 1971; Gerring *et al.*, 1981; Soma *et al.*, 1983; Houston *et al.*, 1985); however, to the authors' knowledge, the exact metabolic enzymes responsible for metabolism of the two compounds in the horse have yet to be determined. While further study, specifically to determine the metabolic enzymes responsible for metabolism of each, would be prudent to fully describe potential drug–drug interactions, this was beyond the scope of the current study. Results of the current study, in contrast to what was reported by Robinson and colleagues (2014), suggest that PBZ does not interfere with the elimination of MCBL *in vivo*.

The current study describes the pharmacokinetics of MCBL following administration of doses that are higher than those

previously studied as well as following multiple-dose administration. In addition, we sought to test the hypothesis that administration of MCBL and PBZ in close proximity to each other would increase the detection time of MCBL. Results of this study suggest that a prolonged withdrawal time is necessary when doses in excess of 5 g are administered to performance horses but that concurrent PBZ administration will not interfere with MCBL clearance.

ACKNOWLEDGMENTS

Financial support for this project was provided by the California Department of Food and Agriculture's Equine Medication Monitoring Program. The authors would like to thank Stacy Steinmetz, Alex White, Nadia Chapman, and Sandy Yim for technical assistance.

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