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Pharmacokinetics and pharmacodynamics of glycopyrrolate following a continuous-rate infusion in the horse

M. J. RUMPLER*
B. KANDALA†
T. W. VICKROY*
G. HOCHHAUS† &
R. A. SAMS‡

*Department of Physiological Sciences, University of Florida College of Veterinary Medicine, Gainesville, FL, USA; †Department of Pharmaceutics, University of Florida College of Pharmacy, Gainesville, FL, USA; ‡HFL Sport Science, Inc., Lexington, KY, USA

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Glycopyrrolate (GLY) is an antimuscarinic agent that is used in humans and domestic animals primarily to reduce respiratory tract secretions during anesthesia and to reverse intra-operative bradycardia. Although GLY is used routinely in veterinary patients, there is limited information regarding its pharmacokinetic (PK) and pharmacodynamic (PD) properties in domestic animals, and an improved understanding of the plasma concentration–effect relationship in racehorses is warranted. To accomplish this, we characterize the pharmacokinetic–pharmacodynamic (PK-PD) actions of GLY during and after a 2-h constant-rate intravenous infusion (4 μg/kg/h) and evaluate potential PK-PD models for cardiac stimulation in adult horses. Measurements of plasma GLY concentrations, heart and respiration rates, and frequency of bowel movements were performed in six Thoroughbred horses. The time course for GLY disposition in plasma followed a tri-exponential equation characterized by rapid disappearance of GLY from blood followed by a prolonged terminal phase. Physiological monitoring revealed significant ($P < 0.01$) increases in heart (>70 bpm) and respiratory rates accompanied by a marked and sustained delay in the frequency of bowel movements (1.1 ± 0.2 h [saline group] vs. 6.0 ± 2.0 h [GLY group]). Two of six horses showed signs of colic during the 8-h observation period after the end of the GLY infusion, but were treated and recovered without further complications. The relationship between plasma GLY concentration and heart rate exhibited counterclockwise hysteresis that was adequately described using an effect compartment.

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Marc J. Rumpler, Department Pathology, Immunology and Laboratory Medicine, College of Medicine, University of Florida, 4800 SW 35th Dr., Gainesville, FL 32608, USA. E-mail: mrumpler@ufl.edu

INTRODUCTION

Glycopyrrolate (GLY) is a synthetic muscarinic anticholinergic agent that was identified through efforts to develop candidate anticholinergic agents with a diminished capacity to reach central nervous system (CNS) tissues (Franko & Lunsford, 1960). In the case of GLY, the goal to develop an agent with markedly reduced CNS effects was accomplished by incorporation of a quaternary ammonium substituent that retains a permanent positive charge, whereas naturally occurring analogues such as atropine contain the more lipophilic tertiary amine substituent. Aside from differences in the CNS actions, the spectrum of pharmacological actions by GLY is qualitatively similar to that of the naturally occurring alkaloids atropine and scopolamine, but differs with regard to duration and intensity. Within the peripheral nervous system, GLY acts as a potent competitive antagonist at muscarinic receptors and attenuates physiological processes regulated by the parasympathetic nervous system, including predictable actions within the respiratory tract, gastrointestinal system, and heart (Barocelli *et al.*, 1993). The pronounced actions of GLY in cardiac tissues provide a basis for its clinical use in veterinary patients for pharmacological reversal of vagally mediated bradycardia in domestic large (Singh *et al.*, 1997; Teixeira Neto *et al.*, 2004a,b) and small (Short & Miller, 1978; Dyson *et al.*, 1999) animal species while under general anesthesia.

The pharmacokinetics (PKs) and pharmacodynamics (PDs) of GLY and other anticholinergic agents have been extensively studied in humans (Gal & Suratt, 1981; Gal *et al.*, 1984; Alimelkkila *et al.*, 1989, 1990, 1991, 1993), but are less well

characterized in domestic non-human species. For example, several descriptive reports of GLY actions in horses have been published (Singh *et al.*, 1995, 1997; Dyson *et al.*, 1999; Teixeira Neto *et al.*, 2004a,b), although, to date, there has been no systematic evaluation of the combined pharmacokinetic–pharmacodynamic (PK-PD) properties of this drug in horses. Nevertheless, there is need for a thorough understanding of the PK-PD relationship for GLY in performance horses in view of its designation as a potential performance-altering substance as evidenced by its classification as a Class 3 agent by the Association of Racing Commissioners International (ARCI; Short *et al.*, 1998).

Our laboratory has developed and fully validated a robust and highly sensitive analytical method for quantification of GLY in horse plasma (Rumpler *et al.*, 2011b). Using that analytical method, we have conducted a detailed characterization of GLY PK properties in Thoroughbred horses after a single intravenous dose administration (Rumpler *et al.*, 2011a). Results from the PK analysis demonstrated that GLY is characterized by a comparatively large steady-state volume of distribution and is cleared very rapidly after a rapid intravenous dose administration, thereby making a detailed quantitative analysis of the drug's physiological effects difficult due to rapidly changing plasma concentrations. As an alternative approach, we have administered the drug by constant-rate infusion (CRI) as a means to enable better characterization of PK–PD relationships for drugs that undergo rapid distribution and/or elimination (Derendorf & Hochhaus, 1995). In this study, GLY was administered to adult Thoroughbred horses by CRI (4 μg/kg/h for 2 h), and the resultant relationships between plasma drug concentration and several physiological measures were evaluated.

MATERIALS AND METHODS

Animals

Six adult Thoroughbred geldings ranging in age from 9 to 11 years and weighing 540–595 kg were used in this study. All horses were determined to be healthy before enrollment in the study based on physical examination, complete blood count (CBC), horse blood chemistry panel, urine analysis, indirect blood pressure measurement, and electrocardiography (ECG). One week before the start of the study, pairs of subjects underwent three separate 5-h sham treatments in order to acclimate them to the experimental protocol, including equipment, experimental procedures, and handling, in an attempt to minimize the effect of behavioral stressors or unique environmental conditions associated with the actual drug treatment. On the morning of the study, horses were weighed and allowed to feed on a commercially available grain mixture up to 2 h before drug administration. Each horse was housed indoors at the University of Florida (UF) Veterinary Medical Center, unrestrained, in an individual climate-controlled (26 °C) stable throughout the dosing and direct observation period, and had open access to water at all times. Following the direct observation period, horses were released outdoors to grass paddocks. The experimental protocol, including drug administration and sample collection, was approved, and facilities were inspected periodically by the UF Institutional Animal Care and Use Committee.

Dosing and collection

The study consisted of a randomized two-way crossover design wherein each of the six horses received a CRI of GLY (4 μg/kg/h) and a saline (0.9% NaCl) control with a 10-day washout period between treatments, thereby allowing for each horse to serve as its own control. On the days during which drug administration took place, the study always began at 08:00 to reduce variability associated with circadian rhythm changes. Baseline observations took place for 1 h (08 00–09 00) before drug administration. Following the baseline observation period, horses were administered GLY (glycopyrronium bromide; American Regent, Inc., Shirley, NY, USA) using local lidocaine anesthesia and a 14-gauge catheter aseptically placed into the right jugular vein, at an intravenous CRI (Medex 3010, Duluth, GA, USA) of 4 μg/kg/h for 2 h yielding a total drug dose of 8 μg/kg. This dose was based on a previous PK analysis (Rumpler *et al.*, 2011a) in a similar group of horses and was intended to achieve a steady-state concentration. After the end of the infusion period, all horses remained in the stall and were observed directly until the first bowel movement. After a satisfactory general health assessment for abdominal discomfort and signs of colic, the horses were returned to outdoor paddocks.

Whole blood samples were collected from the left jugular vein via needle venipuncture into partially evacuated tubes containing lithium–heparin. Whole-blood samples were stored on ice until the plasma was concentrated by centrifugation (776–1318 ***g***) at 4 °C for 15 min. Centrifugation took place within 1 h of sample collection, and 2–4-mL aliquots of plasma were frozen immediately at −20 °C and stored within 24 h at −80 °C until analyzed. Collection times were recorded relative to the start of the infusion and included a sample collection before drug administration and at 5, 10, 15, 20, 30, 45, 60, 90, 120 (end of infusion), 122.5, 125, 130, 135, 140, 150, 165 min, and 3, 3.5, 4, 5, 6, 8, 10, 14, and 26 h after the start of the infusion. Samples were stored for no longer than 6 weeks at −80 °C (4 weeks) and −20 °C (2 weeks), conditions which were well within the validated stability limitations previously reported (Rumpler *et al.*, 2011b).

Determination of protein binding

One hundred milliliters of venous blood was collected from each of the six horses, aged 3–8 years, into tubes containing lithium–heparin (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ, USA). Each horse had been drug-free for the previous 30 days and was considered healthy, based upon physical examination, complete blood count, serum chemistry analysis, and plasma fibrinogen concentration. As extended storage of

plasma has been shown to affect the plasma protein binding (PB) of certain drugs (Paxton, 1981), plasma was freshly harvested within 1 h of blood collection through centrifugation (15 min at 2000 ***g***) of the blood samples. All plasma was pooled, and the pH was adjusted from 7.3 to 7.4. The extent of GLY plasma PB was determined by ultrafiltration (UF) methodology as described previously (Sebille *et al.*, 1990a, 1990b; Wright *et al.*, 1996).

Calibrators were prepared using the freshly harvested plasma for this experiment. A set of positive control samples was prepared from fresh plasma, and a set of protein-free negative control samples was prepared in phosphate-buffered saline (pH 7.4), to achieve end concentrations of 25, 10, 5, 1, 0.5, and 0.1 ng/mL. A 1-mL aliquot of each plasma dilution and of the negative control solution was then transferred into a CryoTube™ vial (Nunc, Roskilde, Denmark), incubated at 37 °C for 20 min, flash-frozen in liquid nitrogen, and placed in a freezer at −20 °C until analyzed. A second 1-mL aliquot of each sample was transferred into the sample reservoir of the Centrifree® ultrafiltration device (Millipore Corp, Bedford, MA, USA) and incubated at 37 °C for 10 min to allow drug–plasma PB equilibrium. The samples were then centrifuged at 2000 ***g*** and 37 °C for 15 min. After centrifugation, the filtrate cup was disconnected from the filtration device, sealed with a cap, flash-frozen, and stored in a freezer at −20 °C until analyzed.

The concentration of protein-bound drug (C_b) was calculated as follows:

$$C_b = C_t - C_f, \quad (1)$$

where C_t is the total GLY concentration, and C_f is the concentration of the free fraction of GLY. The percentage plasma PB was calculated as follows:

$$\mathrm{PB} = C_b/C_t \times 100 \quad (2)$$

Similarly, the degree of nonspecific adsorption (A) of GLY to the filtration device was determined based on the phosphate-buffered saline diluted and protein-free negative control samples as follows:

$$A = C_b/C_t \times 100 \quad (3)$$

Determination of plasma glycopyrrolate concentrations

Plasma GLY concentrations were determined using a fully validated ultra-high-performance liquid chromatography (UHPLC) and tandem mass spectrometry (MS/MS) method as previously described (Rumpler *et al.*, 2011b) and in accordance with US FDA-recommended guidelines (Guidance for Industry, 2001) for bioanalytical methods. The method was characterized by a limit of detection (LOD) and lower limit of quantitation (LLOQ) of 0.025 and 0.125 pg/mL in plasma and was deemed suitable for this study.

Pharmacokinetic analysis

Nonlinear least-squares analysis was performed on plasma GLY concentration vs. time data, and PK parameters for all horses were estimated with compartmental analysis using Phoenix WinNonlin® 6.1 (Pharsight, St. Louis, MO, USA). The Gauss–Newton (Levenberg and Hartley) method was used, and goodness of fit and the appropriate weighting factor were selected based on the coefficients of variation, Akaike information criterion (AIC; Yamaoka *et al.*, 1978), and Schwarz's Bayesian (Schwarz, 1978) Criteria (SBC) as well as visual analysis of the graphical output (including residual plots). Secondary parameters included area under the curve (AUC), terminal half-life ($t_{1/2\gamma}$), absolute volumes of distribution, total plasma clearance (Cl_p), and microdistribution rate constants. All calculations for PK parameters were based on the methods described by Gibaldi and Perrier (1982). The plasma drug concentration at steady-state (C_{pss}) was calculated as follows:

$$C_{pss} = R_o/Cl_p, \quad (4)$$

where R_o is the drug infusion rate, and Cl_p is the systemic (total) drug clearance. All PK parameters were calculated individually for each horse, and values are reported as median and range (minimum–maximum).

Physiological endpoints

Horses were kept under constant direct observation from 08:00 (1 h before administration) until 13:00 (2 h following the end of the infusion). Any clinical signs attributable to drug response were carefully monitored and recorded. Heart rate and respiratory rate were recorded every 10 min for 1 h before drug administration and again every 10 min until 4 h after the start of the infusion. Heart rate was measured using a telemetric device fastened with a girth for continuous monitoring. Respiratory rate was determined by direct observation.

Defecation, micturition incidence, and stool consistency were recorded throughout the observation period. Horses were retained in indoor stalls until the first bowel movement after the end of drug administration was recorded. Following a routine health evaluation, horses were turned out to pasture. In addition to direct observation, all horses were video-recorded from 08:00 (1 h before administration) until 13:00 (2 h after administration) for a total of 5 h. Abnormal behavior was evaluated based on a detailed rubric (Price *et al.*, 2003).

PK-PD modeling

The relationship between plasma GLY concentrations and PD effect was assessed for heart rate. PK-PD linked analysis was performed using Phoenix WinNonlin® 6.1 (Pharsight). The model was selected based on AIC, SBC, and a visual inspection of the fitted plot of concentration vs. time for each horse. Minimum AIC estimates were applied to discriminate the best fitting model, and improved fit of data was achieved by reweighting.

Statistical analysis

Plasma GLY concentrations are reported as mean ± standard deviation, and PK parameter estimates are reported as median values with the range (minimum–maximum) for individual subjects. Differences in plasma PB between different nominal GLY concentrations were assessed using one-way repeated-measures ANOVA with Tukey's post hoc test. Differences between treatment and control groups for physiological endpoints were assessed using two-way ANOVA with Bonferroni's post hoc test. All analyses were performed using commercial statistical software (Microsoft® Office Excel 2003; Microsoft Corporation, Redmond, WA, USA; and GraphPad Prism™ version 5.0 for Windows [GraphPad Software, San Diego, CA, USA]). Any P value < 0.05 was considered to be statistically significant.

RESULTS

Protein binding

The fraction of GLY bound to plasma protein over a range of plasma drug concentrations (0.1–25 ng/mL) was 37–44% (Table 1). The extent of nonspecific adsorption was −0.9% to 2.9%, indicating that adsorption of GLY to the filtration apparatus was negligible.

GLY pharmacokinetics

Plasma GLY concentrations were determined for 24 h following a 2-h constant-rate intravenous infusion of the drug for a total of 26 h. Median results for the PK estimates in the six treated horses are reported in Table 2. Following discontinuation of the 2-h CRI (4 μg/kg/h), plasma GLY concentrations dropped rapidly in all subjects and followed a time course that was well described by a three-compartment exponential model. The equation for this model based on macro constants is as follows:

$$C_t = A(\exp^{-\alpha \cdot T} - \exp^{-\alpha \cdot t}) + B(\exp^{-\beta \cdot T} - \exp^{-\beta \cdot t}) + C(\exp^{-\gamma \cdot T} - \exp^{-\gamma \cdot t}), \quad (5)$$

where C_t is the plasma concentration at time (t); A, B, and C are the y-axis intercepts for the first, second, and third phases, respectively; α, β, and γ are the exponential terms for each corresponding phase; T is the duration of infusion, and exp is the base of the natural logarithm (Gabrielsson & Weiner, 2007). The weighting factor used for this model was $1/(Y^2)$, where Y is the observed plasma concentration.

Table 2. Mean plasma glycopyrrolate (GLY) concentrations during and after an intravenous constant-rate infusion of GLY in six adult Thoroughbred horses

Time (h)	Mean ± SD (ng/mL)	Minimum (ng/mL)	Maximum (ng/mL)
0	<LOD	<LOD	<LOD
0.08	1.38 ± 0.209	1.06	1.63
0.17	2.94 ± 0.131	2.77	3.08
0.33	3.85 ± 0.278	3.47	4.20
0.5	4.19 ± 0.461	3.60	4.73
0.67	4.53 ± 0.496	3.92	5.14
1	4.95 ± 0.498	4.28	5.49
1.5	5.39 ± 0.581	4.66	6.06
2	5.47 ± 0.584	4.72	6.04
2.04	4.10 ± 1.12	2.72	5.45
2.08	3.45 ± 0.957	2.17	4.91
2.17	2.06 ± 0.693	1.26	2.98
2.25	1.37 ± 0.538	0.76	2.11
2.33	0.911 ± 0.316	0.531	1.29
2.5	0.569 ± 0.234	0.276	0.896
2.75	0.360 ± 0.160	0.165	0.616
3	0.247 ± 0.125	0.112	0.463
3.5	0.134 ± 0.059	0.053	0.214
4	0.092 ± 0.040	0.041	0.147
5	0.048 ± 0.023	0.021	0.082
6	0.032 ± 0.013	0.017	0.051
8	0.019 ± 0.007	0.012	0.030
10	0.013 ± 0.005	0.008	0.021
14	0.007 ± 0.003	0.004	0.011
26	0.005 ± 0.002	0.002	0.008

A plot of the mean plasma GLY concentrations vs. time is shown (Fig. 1). The inset graph demonstrates the approach to steady-state conditions. Observed values as well as model-derived results for individual subjects are shown in Fig. 2). PK parameter estimates derived by compartmental model analysis are presented in Table 3. The initial disposition phase was characterized by a median half-life ($t_{1/2\alpha}$) of 0.12 h (7.2 min) as well as intermediate ($t_{1/2\beta}$) and terminal ($t_{1/2\gamma}$) disposition phases of 0.78 h and 13.2 h, respectively. The median (minimum–maximum) plasma GLY concentration at the end of infusion was 5.10 (3.90–6.18) ng/mL.

Physiological measurements

In conjunction with PK studies, each horse was monitored for changes in heart rate, respiratory rate, and defecation frequency during and following CRI of saline or GLY. A composite plot of the time course and magnitude of changes in heart rate for the group is presented in Fig. 3. During the 1-h period pre-

Table 1. Percent protein binding (mean ± SD) of glycopyrrolate (GLY) in the plasma of six (n = 6) healthy horses

	Nominal plasma GLY concentration (ng/mL)					
	0.1	0.5	1	5	10	25
Protein binding (%)	42.9 ± 8.9	43.5 ± 3.4	44.1 ± 2.1	37.3 ± 3.2	40.1 ± 5.8	37.5 ± 7.5
Nonspecific adsorption (%)	1.5 ± 2.2	2.3 ± 1.9	0.9 ± 1.1	3.3 ± 3.6	2.9 ± 1.1	1.8 ± 1.4

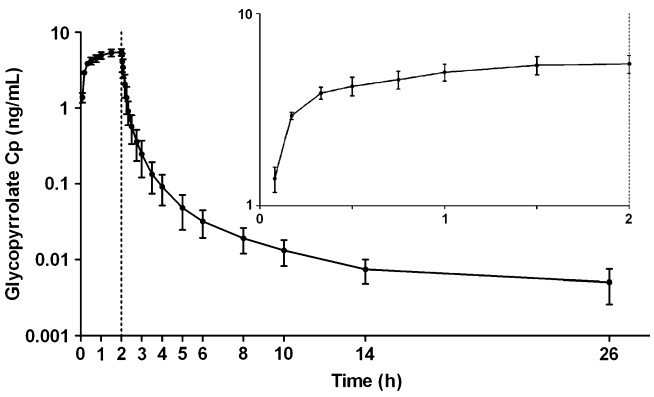

Fig. 1. Plasma glycopyrrolate concentration vs. time curve after a 2-h constant-rate infusion from 0 to 26 h displaying the mean ± SD for all six subjects. The inset details the infusion period.

ceding CRI treatment, resting heart rate for the six subjects averaged approximately 40 beats per min and was not altered by saline infusion. In response to CRI of GLY (4 μg/kg/h), heart rates of all subjects increased ($P < 0.01$) within 50 min following the start of drug infusion. Mean heart rates continued to increase ($P < 0.001$) to a peak average value of >70 beats/min and remained at that level until drug infusion was terminated. Mean heart rates remained high for 40 min after the discontinuation of the infusion and returned to pretreatment values by 90 min postinfusion.

Respiratory rates (expressed as breaths per min) were monitored throughout the direct observation period for GLY, and saline infusions and composite results for all subjects are presented in Fig. 4. The mean respiratory rate of the GLY-treated group was high compared with the control group beginning approximately 20 min after the start of CRI and remained elevated until approximately 20 min before the completion of GLY infusion. Respiratory rates varied substantially among subjects during and after CRI with one subject (Horse 3) showing no appreciable response to GLY infusion (Fig. 4).

The incidence and frequency of bowel movements were recorded in all subjects as a measure of potential gastrointestinal changes associated with GLY infusion. GLY-treated horses exhibited a marked reduction in the total number of bowel movements from 5.0 ± 1.0 (saline CRI) to 1.0 ± 0.2 (GLY CRI; Fig. 5) during the period from 1 h before until 2 h following CRI. A temporal plot of bowel movements during each hourly period reveals that the inhibitory effect of GLY was absolute during the second hour of drug infusion as well as during the first 2 h following termination of GLY treatment. The average time to first bowel movement following termination of CRI treatment was 1.1 ± 0.2 h (saline group) vs. 6.0 ± 2.0 h (GLY group) with one horse exhibiting a 9-h delay in defecation.

Behavioral variation ranged from circling the stall continuously to standing still. Throughout the study, all horses showed mild behavioral changes in the form of shifting weight from one leg to the other and occasional muscle fasciculations.

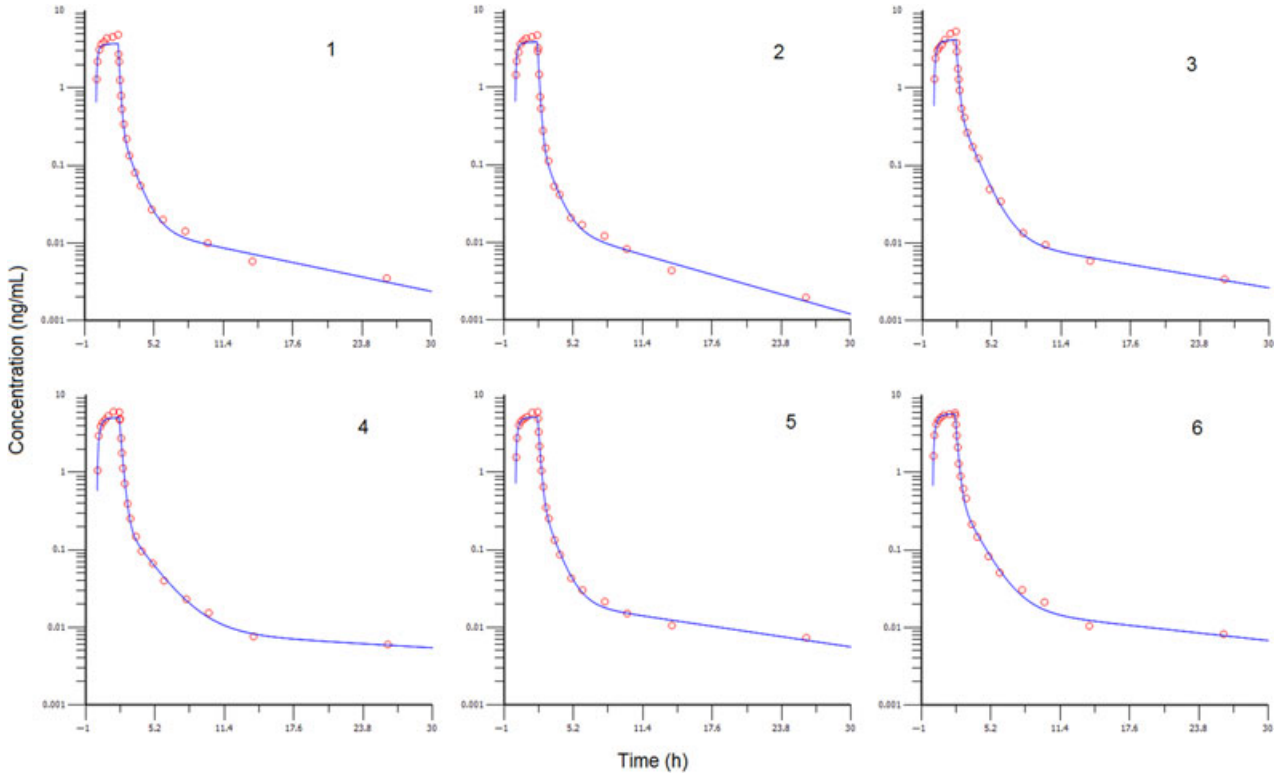

Fig. 2. Observed (circles) and predicted (line) plasma glycopyrrolate (GLY) concentrations after a 2-h intravenous infusion of 4 μg/kg/h of GLY to six ($n = 6$) healthy adult Thoroughbred horses and pharmacokinetic analysis using a three-compartment model.

Table 3. Pharmacokinetic parameter estimates of glycopyrrolate (GLY), determined using a three-compartmental model, following a 2-h intravenous constant-rate infusion of GLY (4 μg/kg/h) to six (n = 6) healthy adult thoroughbred horses

Parameter	Subject #								
	1	2	3	4	5	6	Median	Min.	Max.
A (ng/mL)	47.7	47.7	41.6	40.8	51.0	47.3	47.5	40.8	51.0
B (ng/mL)	0.806	0.556	1.13	0.367	1.19	0.928	0.867	0.367	1.19
C (ng/mL)	0.018	0.018	0.013	0.010	0.023	0.019	0.018	0.010	0.023
Alpha (per h)	7.03	6.59	5.73	4.22	5.50	4.56	5.61	4.22	7.03
Beta (per h)	1.05	1.071	0.822	0.473	0.995	0.650	0.908	0.473	1.07
Gamma (per h)	0.072	0.095	0.056	0.019	0.050	0.036	0.053	0.019	0.095
C_{max} (ng/mL)	3.74	3.86	4.20	5.08	5.17	5.73	4.64	3.74	5.73
V_1 (L/kg)	0.165	0.166	0.187	0.194	0.153	0.166	0.166	0.153	0.194
K_{21} (per h)	1.150	1.135	0.952	0.507	1.10	0.725	1.02	0.507	1.15
K_{31} (per h)	0.075	0.097	0.057	0.020	0.052	0.038	0.055	0.020	0.097
K_{10} (per h)	6.22	6.07	4.82	3.76	4.77	3.91	4.80	3.76	6.22
K_{12} (per h)	0.511	0.308	0.650	0.246	0.413	0.399	0.406	0.246	0.650
K_{13} (per h)	0.203	0.147	0.127	0.175	0.210	0.172	0.173	0.127	0.210
K_{10}_HL (h)	0.112	0.114	0.144	0.184	0.145	0.177	0.145	0.112	0.184
$t_{1/2\alpha}$ (h)	0.099	0.105	0.121	0.164	0.126	0.152	0.124	0.099	0.164
$t_{1/2\beta}$ (h)	0.660	0.647	0.843	1.47	0.697	1.07	0.770	0.647	1.47
$t_{1/2\gamma}$ (h)	9.59	7.32	12.4	35.8	14.0	19.1	13.2	7.32	35.8
AUC_{0-24} (h*ng/mL)	7.80	7.94	8.88	10.9	10.9	12.3	9.91	7.80	12.3
Cl_p (mL/min/kg)	17.1	14.1	15.0	12.2	12.2	10.8	13.6	10.8	17.1
$AUMC_{0-24}$ (h*h*ng/mL)	13.0	5.23	16.1	40.3	23.4	31.5	19.7	11.6	40.3
V_{ss} (L/kg)	0.686	0.462	0.729	1.96	0.831	1.01	0.780	0.462	1.96
V_2 (L/kg)	0.073	0.045	0.128	0.094	0.058	0.091	0.082	0.045	0.128
V_3 (L/kg)	0.448	0.251	0.414	1.68	0.620	0.751	0.534	0.251	1.68
C_{pss} (ng/mL)	3.90	4.73	4.45	5.48	5.48	6.18	5.10	3.90	6.18

A, B, and C, intercepts at t = 0 for the model equation; alpha, beta, and gamma, slopes for the model equation; C_{max}, extrapolated plasma GLY concentration at time 0; V_1, V_2, and V_3, volumes of the central, second, and third compartments, respectively; k_{21}, k_{31}, k_{12}, and k_{13}, distribution rate constants; k_{10}, elimination rate constant; $t_{1/2\alpha}$, phase 1 half-life; $t_{1/2\beta}$, phase 2 half-life; $t_{1/2\gamma}$, phase 3 half-life; AUC, area under the plasma concentration vs. time curve; Cl_P, total plasma clearance; AUMC, area under the first moment curve; V_{ss}, volume of distribution at steady-state; C_{pss}, plasma GLY concentration at steady-state.

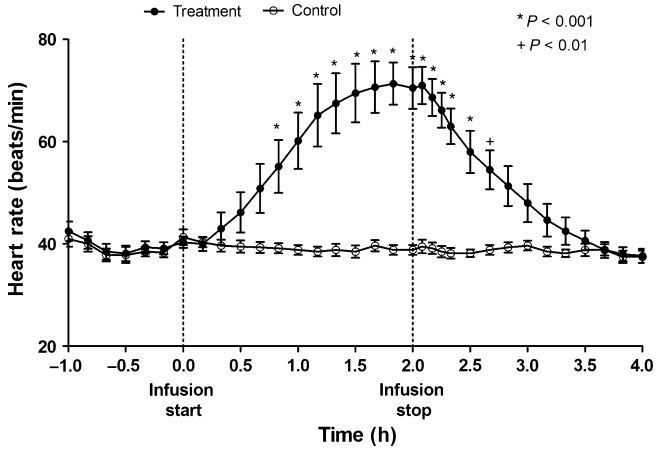

Fig. 3. Mean (SD) heart rate (bpm) for six horses during and after a 2-h constant-rate infusion of glycopyrrolate (GLY) (●) or saline (○). Asterisk (*) and + indicate a significant difference between GLY vs. saline treatments.

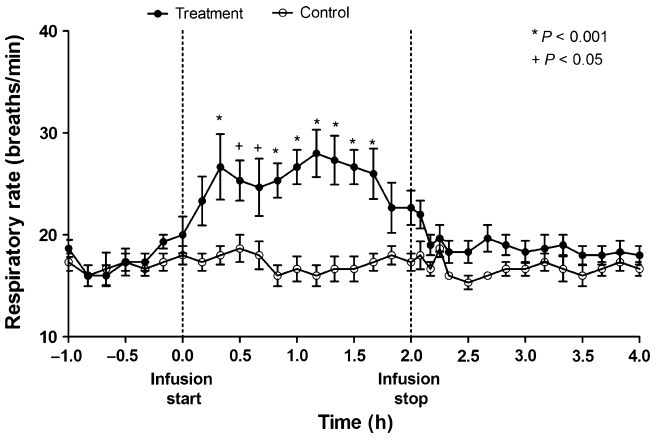

Fig. 4. Mean (SD) respiratory rate (breaths/min) for six horses during and after a 2-h constant-rate infusion of glycopyrrolate (GLY) (●) or saline (○). Asterisk (*) and + indicate a significant difference between GLY vs. saline treatments.

During the study, it was observed that all treated horses exhibited a loss of appetite and refused treats within 60 min after the start of the infusion, while control horses readily accepted them throughout the treatment period. Several hours after drug administration, two GLY-treated horses showed signs of colic and were treated with flunixin meglumine.

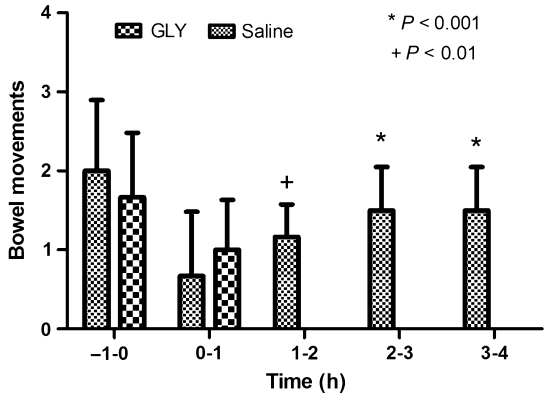

Fig. 5. Mean (+SD) number of bowel movements for saline-treated vs. Glycopyrrolate (GLY)-treated horses (n = 6) during each 1-h period of the direct observation period. Infusion of saline or GLY occurred from 0 to 2 h. There was a complete absence of bowel movements in GLY-treated subjects during hours 1–4.

Temporal relationships between plasma GLY concentration and cardiorespiratory changes: PK-PD modeling

Temporal plots of plasma GLY concentration vs. heart rate and respiratory rate are shown in Fig. 6. When plasma drug concentrations were plotted against the corresponding heart rate effects, a counterclockwise hysteresis loop was observed, indicating a negative temporal displacement between the PD effect (tachycardia) and plasma GLY concentration (Fig. 6a). To accommodate this temporal disconnect, the model chosen included an effect compartment, and the relationship between the effect compartment concentration and response was assumed to be linear (Sheiner *et al.*, 1979). Effect compartment modeling with heart rate effects following GLY administration was performed using a sigmoidal E_{max} model with baseline effect, in which the individual PK parameter estimates determined were used as constants. The equation for the effect-site concentration (C_e) during and after the constant GLY infusion is as follows

$$C_e = \frac{k_0 \cdot k_{e0}}{V_c} \cdot \left[\begin{array}{l} \frac{(k_{21}-\lambda_1)(k_{31}-\lambda_1)(1-e^{-\lambda_1 \cdot T}) \cdot e^{-\lambda_1 \cdot t}}{\lambda_1(\lambda_2-\lambda_1)(\lambda_3-\lambda_1)(k_{e0}-\lambda_1)} + \frac{(k_{21}-\lambda_2)(k_{31}-\lambda_2)(1-e^{-\lambda_2 \cdot T}) \cdot e^{-\lambda_2 \cdot t}}{\lambda_2(\lambda_1-\lambda_2)(\lambda_3-\lambda_2)(k_{e0}-\lambda_2)} \\ + \frac{(k_{21}-\lambda_3)(k_{31}-\lambda_3)(1-e^{-\lambda_3 \cdot T}) \cdot e^{-\lambda_3 \cdot t}}{\lambda_3(\lambda_1-\lambda_3)(\lambda_2-\lambda_3)(k_{e0}-\lambda_3)} + \frac{(k_{21}-k_{e0})(k_{31}-k_{e0})(1-e^{-k_{e0} \cdot T}) \cdot e^{-k_{e0} \cdot t}}{k_{e0}(\lambda_2-k_{e0})(\lambda_3-k_{e0})(\lambda_3-k_{e0})} \end{array} \right] \quad (6)$$

where k_0 is the zero-order infusion rate, k_{e0} is the elimination rate constant from the hypothetical effect compartment, and V_c, K_{21}, K_{31}, λ_1, λ_2, and λ_3 are the modeled PK parameters, T is the elapsed time during the infusion (after the infusion T = 2 h), and t is time after the end of the infusion (Coburn, 1981).

$$E = E_0 + \frac{E_{max} \times C_e^n}{EC_{50}^n + C_e^n} \quad (7)$$

Equations 6 and 7 were modeled simultaneously, which provided estimates of the baseline effect (E_0), maximal drug effect (E_{max}), concentration producing 50% of E_{max} (EC_{50}), sigmoidicity factor (n, Hill coefficient), and k_{e0} for each horse (Holford & Sheiner, 1981).The observed and predicted pharmacological responses during the direct observation period from the proposed PK-PD linked model for each subject are plotted in Fig. 7. Linked model PD estimates are reported in Table 4. Figure 8 illustrates a collapse of the hysteresis curve for all individual horses, indicating that the incorporation of a distributional delay was necessary for model analysis.

DISCUSSION

Although PB of GLY appeared to decrease with increasing concentrations indicating possible drug saturation at PB sites in horses, we have little other evidence of concentration-dependent binding. The degree of plasma PB of GLY determined in this study suggests that the potential for displacement interactions with other drugs is unlikely to be clinically significant. However, we did not specifically investigate the influence of GLY metabolites or other drugs on the PB of GLY. To the author's knowledge, PB of GLY has not previously been reported for any species.

GLY disposition in the horse following a CRI resulted in a rapid decrease in plasma concentrations early and a prolonged terminal phase, although this study did not look at GLY plasma or urine concentrations beyond 24 h following the end of the infusion. This profile was similar to that reported following a single bolus administration in horses (Rumpler *et al.*, 2011a). GLY distributed rapidly from the central to the peripheral compartments as demonstrated by the initial disposition-phase median half-life ($t_{1/2\alpha}$) of 0.12 h (7.2 min). Due to a small central compartment volume of distribution (0.166 (0.153–0.194) L/kg) and much higher third compartment volume of distribution (0.534 (0.251–1.68 L/kg), it is thought that the terminal phase represents a redistribution of the drug back to the central compartment. Compared with single intravenous injections, GLY plasma concentration vs. time curves demonstrated little variability between subjects after a continuous-rate intravenous infusion.

Although a three-compartment model was used to characterize the entire plasma GLY concentration vs. time curve, a two-compartment model was used to generate the PK parameter estimates used as link parameters in the PK-PD linked model. This was done because predicted values between two- and three-compartment models from 0 to 4 h of the plasma GLY concentration vs. time curve were nearly identical. It can also be seen from the plots that steady-state conditions have not been achieved. Thus, for these horses, the rate of elimination never reached the rate of infusion. Moreover, as steady-

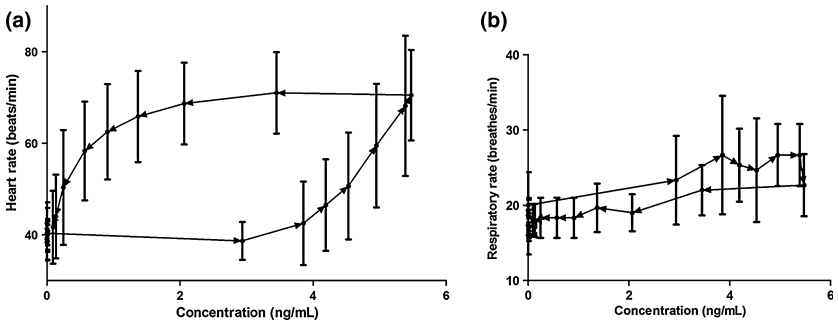

Fig. 6. Temporal relationships between heart rate (a) and respiratory rate (b) vs. plasma glycopyrrolate concentration demonstrating a counter-clockwise hysteresis and clockwise hysteresis, respectively.

Table 4. Pharmacodynamic model parameters (heart rate) for each subject

	Subject #								
Parameter (units)	1	2	3	4	5	6	Median	Minimum	Maximum
E_{max} (bpm)	84.2	67.5	59.9	77.9	80.9	86.0	79.4	59.9	86.0
EC_{50} (ng/mL)	4.52	3.47	4.69	3.28	3.58	1.91	3.53	1.91	4.69
E0 (bpm)	44.5	38.6	33.9	37.9	40.3	41.3	39.5	33.9	44.5
n	2.51	3.47	1.63	3.45	1.25	2.09	2.30	1.25	3.47
k_{e0} (per h)	1.49	0.707	1.54	1.74	0.875	1.65	1.51	0.707	1.74
$t_{1/2}k_{e0}$ (h)	0.465	0.980	0.450	0.398	0.792	0.420	0.458	0.398	0.980

E_{max}, maximal effect; EC_{50}, plasma drug concentration producing 50% of E_{max}; n, Hill coefficient; k_{e0}, rate constant of equilibrium of drug compartment; $t_{1/2}k_{e0}$, half-life of equilibrium of drug in effect compartment.

state conditions are not reached for some of these subjects, the model fails to adequately characterize the observed plasma GLY concentrations near the end of the infusion period.

GLY's effect on heart rate has been previously studied in horses following a 2.5-, 5-, and 10-μg/kg intravenous bolus doses. Mean heart rate (n = 5) after the 2.5-μg/kg dose did not demonstrate a significant difference from the control group for measurements taken up to 120 min. However, the 5- and 10-μg/kg doses both produced elevated mean heart rates compared with the control group beginning 5 min after and ending 60 min after treatment in conscious horses (Singh *et al.*, 1997), in agreement with the results of the current study. In other studies in horses, GLY is used to attenuate the cardiovascular depressive effects of anesthetic agents, such as xylazine. Singh *et al.* (1995) reported that a 2.5-μg/kg dose is effective at reducing atrioventricular bock, but doses such as 5 and 10 μg/kg are associated with a profound loss of gastrointestinal motility and therefore are considered unsafe. Teixeira Neto *et al.* (2004a,b) noted a 53% increase in cardiac output over the control group when horses (n = 6) were intravenously administered 5 μg/kg of GLY and anesthetized with xylazine, while low intestinal auscultation scores were evident. A third study in anesthetized horses reported an increase in mean heart rate after CRI of 5-μg/kg dose intravenously but also warned about unwanted gastrointestinal effects (Dyson *et al.*, 1999).

The current study did not measure overall cardiac output. Yet, it would be expected to increase as a result of the positive chronotropic effects of GLY and other muscarinic antagonists. Further, an increase in cardiac output would likely lead to an increase in total plasma clearance of the drug. In our previous study, the median (range) of total plasma clearance was greater at 22.4 (14.2–31.2) mL/min/kg. Ultimately, the relationship between heart rate and cardiac output would be determined by the magnitude of the change in heart rate (Wessale *et al.*, 1990). In our previous study, we did not document PD measurements.

Respiratory effects in horses following a clinically relevant dose of GLY have not been reported. However, in humans, intravenous GLY has been shown to cause bronchodilation (Gal & Suratt, 1981), while nebulized GLY caused a longer duration of bronchodilation without the systemic anticholinergic effects of inhaled atropine (Gal *et al.*, 1984; Walker *et al.*, 1987). In the horse, GLY would be expected to antagonize muscarinic receptors on the airway smooth muscle and submucosal glands causing bronchodilation and reduced mucus secretions (Coulson & Fryer, 2003). In the current study, PK-PD modeling was not performed using respiratory rate as a physiological parameter. Yet, when plotted, and in contrast to heart rate, a clockwise hysteresis (proteresis) was observed between GLY concentrations and respiratory rate (Fig. 6b). Proteresis generally occurs when a subject develops tolerance for a given compound (Krishna, 2004) and would suggest a PD origin for the delay. Despite the limited value of respiratory rate as an indicator of airway function, differences in respiratory rate were evident between the treatment and control groups.

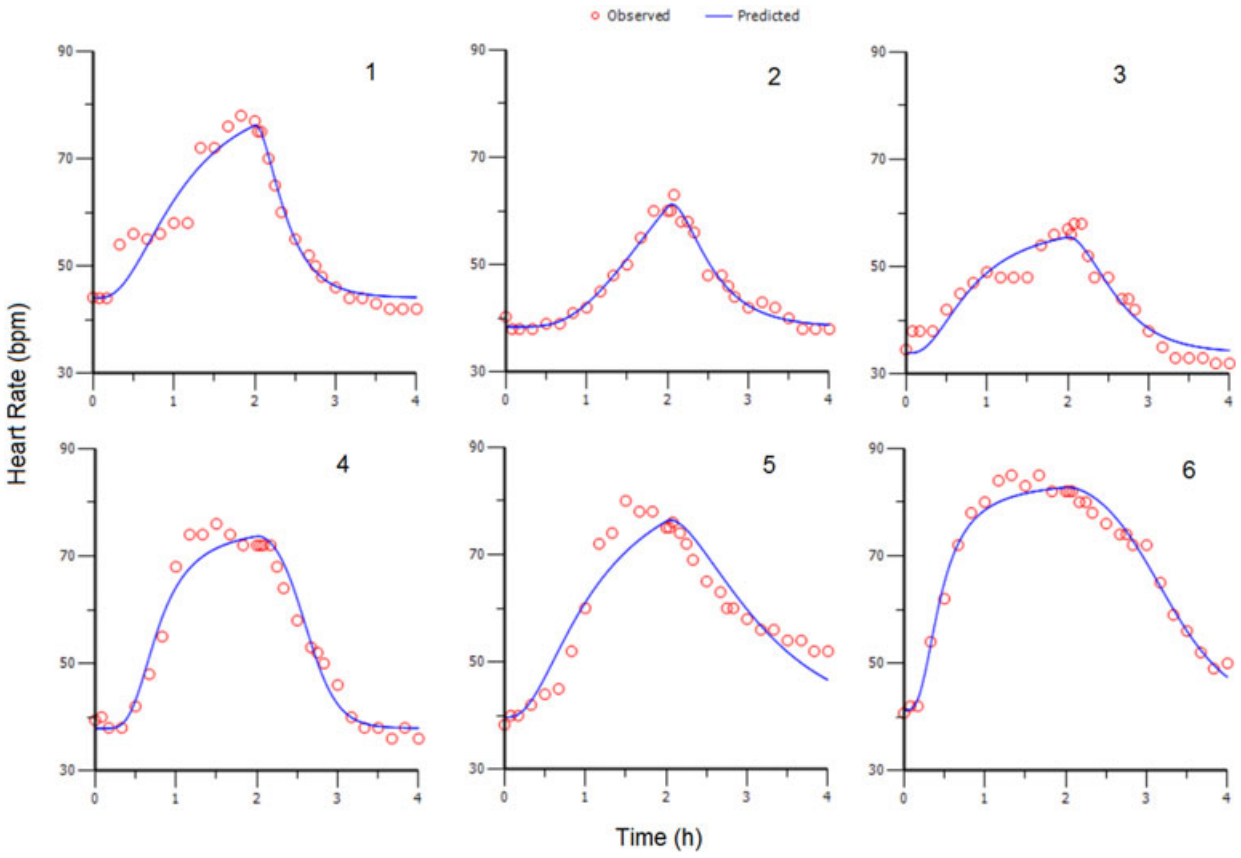

Fig. 7. Pharmacokinetic-pharmacodynamic linked model fit for heart rate for each of the six horses.

The depressant effects of nonselective muscarinic receptor antagonists on intestinal motility have been documented, and the horse is particularly sensitive to the gastrointestinal effects of these compounds. Intestinal smooth muscle contraction occurs via the M_3 receptors located in the gastrointestinal tract (Herdt, 1997). In some cases, GLY has been used successfully to treat vagally mediated bradycardia during anesthesia in small animals (Dyson & James-Davies, 1999) and in goats (Pablo *et al.*, 1995) without causing major complications in other organ systems. In contrast, doses of 5 μg/kg have produced a complete loss of intestinal motility in dogs for up to 6 h (Burger *et al.*, 2006). In horses, doses as low as 5 μg/kg of GLY have resulted in lower auscultation scores and in some cases intestinal impaction and colic (Singh *et al.*, 1995, 1997; Dyson *et al.*, 1999; Teixeira Neto *et al.*, 2004a). According to the current study, it is evident that bowel movements in the treatment group occurred during the pre-administration period and the first hour of the drug infusion, presumably before the drug exerted its effects on the gastrointestinal tract. This study, demonstrated that GLY has the potential to reduce bowel movements and frequency in the period following drug administration compared with control horses administered saline. Although motility was not directly quantified, it is assumed that a reduced fecal frequency and extended periods without defecation are the result of gastrointestinal hypomotility. As such, it has been demonstrated through this and other studies that GLY should be used conservatively at CRI doses below 10 μg/kg, especially in horses with pre-existing gastrointestinal conditions or those undergoing surgery. Doses exceeding 10 μg/kg should not be used in the horse and would likely be associated with severe intestinal complications (Adams, 2001).

This study attempted to evaluate behavioral effects associated with the administration of GLY to the horse, and the subjects were video-monitored for 5 h from the time they were led into the stalls. Although each subject's behavior varied while housed, all subjects appeared more relaxed when a companion animal was housed in an adjacent stall for the duration of the observation period. The loss of appetite likely resulted from a gastrointestinal disturbance, and two horses demonstrated acute signs of colic. These horses were evaluated on the following morning and were found to be in normal condition after an unremarkable examination.

There was obvious hysteresis observed when heart rate and plasma GLY concentrations were plotted against time and when the effect was plotted against plasma GLY concentrations that were minimized by the incorporation of an effect compartment model (Fig. 8). This finding is surprising because muscarinic receptors are predominately located in the heart and GLY concentrations at the effect site are expected to be similar to measured plasma concentrations. While hysteresis appears to be a characteristic of PK-PD modeling of several drugs active

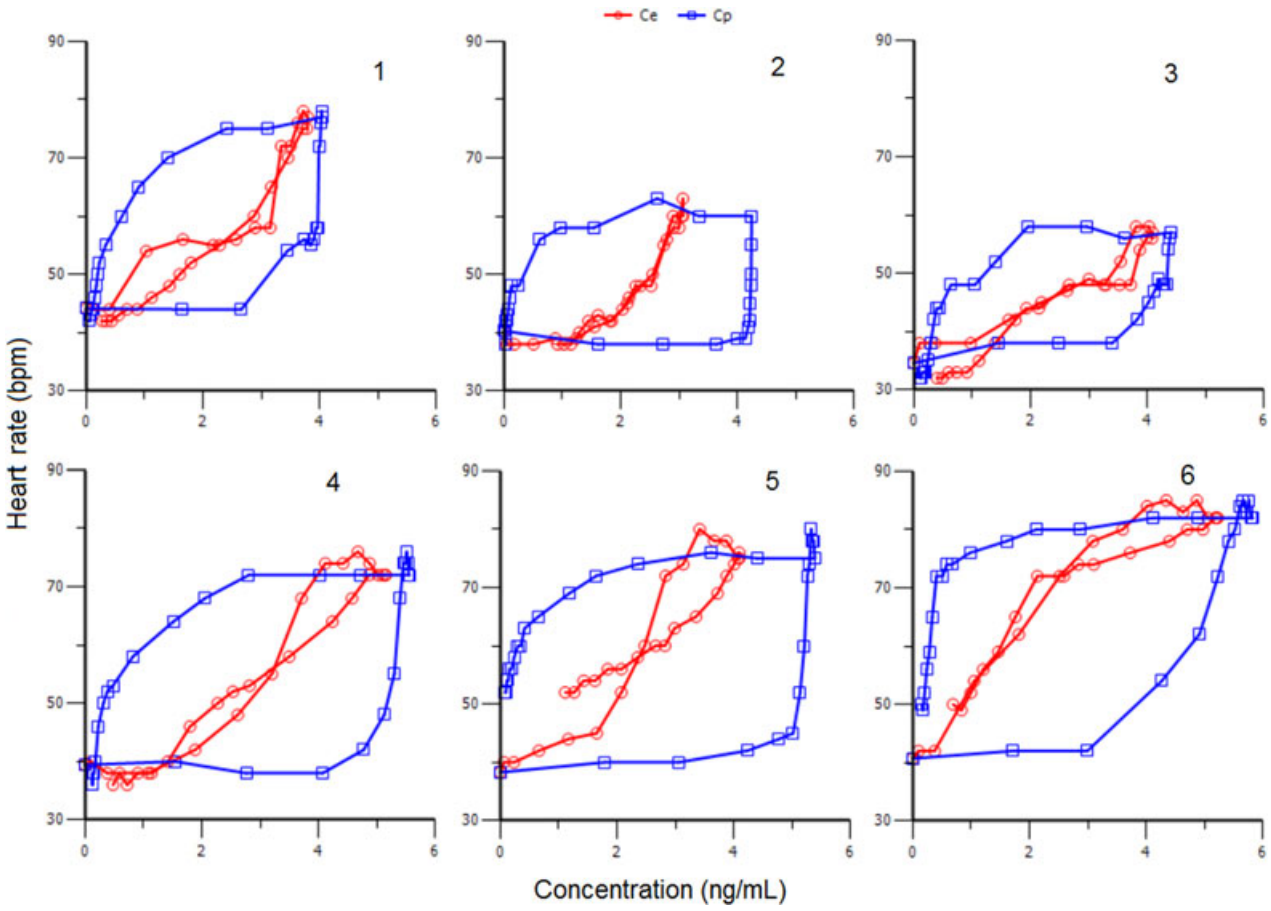

Fig. 8. Individual hysteresis showing the plasma concentration and the predicted effect compartment concentration after the PK-pharmacodynamic linked model was applied.

in the CNS (Mandema *et al.*, 1991; Danhof & Mandema, 1992), the current study does not permit us to establish whether distribution is the major determinant of the observed hysteresis. However, the rationale for this delay is easily understood if one assumes that the resulting pharmacological effect or response is preceded by drug distribution to the site of action. Nonetheless, as GLY poorly permeates the CNS barrier due to its polarity, a temporal delay as a result of drug distribution to these sites is unlikely.

The hysteresis has been successfully modeled by the effect compartment approach, which postulates the existence of a hypothetical effect compartment linked to the plasma site by a first-order process (Sheiner *et al.*, 1979). This approach is based on the assumption that distribution kinetics between plasma and effect site are linear and that the same effect-site concentration always evokes the same response, independent of time. This assumption may not hold in the presence of active metabolites or when there is development of acute tolerance. However, there are no reports indicating that active metabolites of GLY are formed in humans (Kaila *et al.*, 1990) or horses (Matassa *et al.*, 1992). Moreover, there is no evidence of the development of tolerance toward the anticholinergic effects of GLY in these models.

It has been well known that GLY and other competitive muscarinic receptor antagonists prevent the action of ACh on the SA node of the heart (Adams (Ed.) (2001). As a result, physiological responses to parasympathetic (vagal) nerve impulses are thereby attenuated or abolished. Furthermore, as muscarinic receptors are also present in the peripheral vasculature, the effects of GLY on heart rate may account for partial subsequent and measurable effects of the drug on the cardiovascular system. Several vagolytic compounds, including GLY, have been shown to increase heart rate beyond that which occurs in vagotomized dogs (Rigel *et al.*, 1984), indicating that the tachycardia induced by antimuscarinic agents is not solely due to vagal blockade. This 'excess tachycardia' may be a result of ganglionic blockade, yet its mechanisms are not fully understood (Schuil *et al.*, 1981; Chassaing *et al.*, 1982; Rigel & Katona, 1986)

In the case of purely peripheral effects, a more direct link of the plasma PKs to the corresponding PD effect would have been expected. However, the physiological situation may be more complex. A long equilibration half-life can also ensue from a high affinity of the drug for the cardiac tissue leading to prolongation of the (peripheral) effects of GLY on the heart or a mechanism other than vagal blockade could result in

excess tachycardia. Furthermore, heart rate is determined by complex peripheral cardiovascular regulatory systems, and changes in one part of the system, such as muscarinic blockade, will likely cause compensatory changes in the components. For example, the increased HR could be a physiological response to vasodilation that resulted in reduced pressures that were restored by increased cardiac output. Unfortunately, we did not measure blood pressure or cardiac output during this study.

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