JOURNAL OF
Veterinary Pharmacology and Therapeutics

J. vet. Pharmacol. Therap. **37**, 260–268. doi: 10.1111/jvp.12085.

The pharmacokinetics of glycopyrrolate in Standardbred horses

M. J. RUMPLER*
P. COLAHAN† &
R. A. SAMS*,[1]

**Florida Racing Laboratory, Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA;* †*Florida Racing Laboratory, Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA*

Rumpler, M. J., Colahan, P., Sams, R. A. The pharmacokinetics of glycopyrrolate in Standardbred horses. *J. vet. Pharmacol. Therap.* **37**, 260–268.

The disposition of plasma glycopyrrolate (GLY) is characterized by a three-compartment pharmacokinetic model after a 1-mg bolus intravenous dose to Standardbred horses. The median (range) plasma clearance (Clp), volume of distribution of the central compartment (V_1), volume of distribution at steady-state (*V*ss), and area under the plasma concentration–time curve ($AUC_{0\text{-inf}}$) were 16.7 (13.6–21.7) mL/min/kg, 0.167 (0.103–0.215) L/kg, 3.69 (0.640–38.73) L/kg, and 2.58 (2.28–2.88) ng*h/mL, respectively. Renal clearance of GLY was characterized by a median (range) of 2.65 (1.92–3.59) mL/min/kg and represented approximately 11.3–24.7% of the total plasma clearance. As a result of these studies, we conclude that the majority of GLY is cleared through hepatic mechanisms because of the limited extent of renal clearance of GLY and absence of plasma esterase activity on GLY metabolism. Although the disposition of GLY after intravenous administration to Standardbred horses was similar to that in Thoroughbred horses, differences in some pharmacokinetic parameter estimates were evident. Such differences could be attributed to breed differences or study conditions. The research could provide valuable data to support regulatory guidelines for GLY in Standardbred horses.

(Paper received 10 May 2013; accepted for publication 19 September 2013)

Marc Rumpler, Clinical & Forensic Toxicology Laboratory, Department of Pathology, College of Medicine, Gainesville, FL 32608, USA.
E-mail: mrumpler@ufl.edu
[1] Present address: HFL Sport Science, Inc. Lexington KY 40509 USA

INTRODUCTION

Glycopyrrolate (GLY) is classified as a Class 3 controlled therapeutic substance by the Association of Racing Commissioners International (ARCI). As such, the ARCI recognizes that GLY has legitimate use as a therapeutic substance in race horses in training but that it must be discontinued before racing due to its potential to affect a horse's performance during a race. The pharmacokinetics and urinary excretion of GLY in horses have been investigated recently (Rumpler *et al.*, 2011b, 2012), and it has been determined that the renal clearance of unchanged GLY accounts for a small fraction of the total clearance because the estimated total plasma clearance is substantially greater than effective renal plasma flow and approaches estimates of hepatic blood flow in the horse. However, our previous studies did not employ an experimental design that would permit estimation of renal clearance. One common design that permits estimation of renal clearance requires volumetric urine collections for a specified time period following drug administration. Once this is completed, renal clearance may be calculated using several methods, most of which are based on analysis of the rate of drug excretion and the corresponding plasma drug concentration (Rowland & Tozer, 1995).

Plasma esterase activity may also contribute to GLY elimination (Chen & Hsieh, 2005). Compounds containing ester groups, such as GLY, may be particularly susceptible to plasma esterases, a heterogeneous family of enzymes that catalyze the hydrolysis of esters (Satoh *et al.*, 2002). Other studies have indicated a minimal inhibitory effect of GLY on plasma cholinesterases in humans, but the extent of GLY hydrolysis by these or other enzymes has not been investigated (Mirakhur, 1985; Zsigmond *et al.*, 1985). Therefore, the following study was also designed to determine whether plasma hydrolysis of GLY occurs and the extent to which this pathway contributes to GLY clearance in the horse.

In addition, this study uses a different horse breed (Standardbred) from the previous (Thoroughbred) study. This will allow a breed comparison of pharmacokinetic disposition and parameter estimates for GLY. We hypothesize that there are no substantive differences in the disposition of GLY between the two breeds.

© 2013 John Wiley & Sons Ltd

METHODS

Animals

Six healthy adult and athletically conditioned Standardbred horses (one mare and five geldings) ranging in age from 4 to 9 years and weighing from 445 to 510 kg were used in these studies. All horses were fed a diet of commercially available grain mixture and were housed indoors at the UF Veterinary Medical Center in climate-controlled stalls for 1 h before and 24 h after drug administration. From 24 h after administration until the conclusion of the study (168 h), all horses were turned out to grass paddocks and had open access to water and hay at all times. Horses were regularly exercised (3 days/week) before and throughout the duration of the study. The conditioning protocol was previously described in detail (Rumpler *et al.*, 2011a).

Dosing & specimen collection

All horses were administered 1 mg (1.96–2.25 μg/kg) of GLY (glycopyrronium bromide; American Regent, Inc., Shirley, NY, USA) intravenously by venipuncture into the right jugular vein. Whole blood samples were collected by venipuncture at predetermined times after administration from the left jugular vein using the procedures described in Rumpler *et al.*, (2011a). Collection times were before drug administration and at 5, 10, 15, 20, 30, and 45 min and 1, 2, 3, 4, 6, 8, 24, 48, 72, 96, and 168 h after intravenous administration. All horses were trained to urinate on command, and the urine from each horse was collected via the free-catch method into separate, clean, 1-L containers. The total urine volume voided through 24 h was collected. Designated collection intervals were every hour. If a subject produced no urine during a collection interval, the next available specimen was taken, and the time and volume were recorded. Additional urine collections were prepared at 48, 72, 96, and 168 h following administration. Urine specimens were aliquoted into 15-mL sterile, disposable, polypropylene centrifuge tubes and stored at −20 °C immediately and at −80 °C within 48 h.

Resistance of glycopyrrolate to plasma esterases

One hundred milliliters of venous blood was collected from a 3-year-old gelding into tubes containing lithium heparin (BD Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ, USA). The horse had been drug-free for the previous 30 days and was considered healthy, based on physical examination, complete blood count, serum chemistry profile, and plasma fibrinogen concentration.

Fresh harvested plasma was collected within 1 h of blood collection through centrifugation of the blood samples for 15 min at 2000 ***g***. All plasma was pooled, and the pH was adjusted to 7.4, if necessary. Stock and working standard solutions of GLY and GLY-d_3 were prepared according to the procedures outlined in Rumpler *et al.* (2011a) as were calibrators and positive control samples using the fresh plasma collected for this experiment.

At each of fourteen predetermined times, 4 mL of fresh plasma was aliquoted to a CryoTube™ vial (Nunc, Roskilde, Denmark). The fresh plasma samples were fortified with 32 μL of 0.00125 ng/μL of aqueous GLY working standard solution for a final concentration of 10 pg/mL. The zero-time sample was immediately capped and flash frozen using liquid nitrogen and stored at −20 °C until analysis. The remaining samples were capped and incubated in a water bath at 38 °C for 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240, 300, and 360 min, flash frozen in liquid nitrogen upon removal and stored in −20 °C until analyzed. All stability samples were analyzed in duplicate according to the LC-MS/MS methods previously described (Rumpler *et al.*, 2011a).

Determination of plasma and urine GLY concentrations

Plasma and urine GLY concentrations were determined according to fully validated LC-MS/MS methods described previously (Rumpler *et al.*, 2011a, 2012) and using an isotopically labeled internal standard (GLY-d_3; atom 99.9% D, 0% D_0). The plasma method was characterized by a linear range of 0.125–25 pg/mL ($R^2 > 0.998$), a lower limit of quantification of 0.125 pg/mL, and a lower limit of detection of 0.025 pg/mL. The intra- and interbatch precisions were <15% coefficient of variation (CV), and the accuracy ranged between 100 and 105%. The urinary method was characterized by a linear range of 5–2500 pg/mL, a lower limit of quantification of 5 pg/mL, and a limit of detection of 1 pg/mL. The intra and interbatch imprecisions were <10% CV, and accuracy ranged between 94% and 104%.

Determination of plasma and urinary creatinine

Creatinine determinations were performed using a MGC 240 benchtop analyzer (Thermo Scientific, Freemont, CA, USA). The methodology is based on the Jaffe reaction, whereby creatinine concentration is determined using alkaline picrate to form a reddish Janovski complex. The absorbance at 505 nm is measured spectrophotometrically and is directly proportional to the creatinine concentration (Hervey, 1953). Creatinine renal clearance was calculated similar to that of GLY (below) using plasma and urine creatinine measurements and the urine volume collected in each collection period.

Pharmacokinetic analysis

Nonlinear least squares regression analysis was performed on plasma GLY concentration vs. time data, and pharmacokinetic 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's information criterion (AIC) (Akaike, 1976; Yamaoka

© 2013 John Wiley & Sons Ltd

et al., 1978), and Schwarz's Bayesian Criterion (SBC) (Schwarz, 1978) as well as visual analysis of the graphical output (including residual plots). Secondary parameters calculated include area under the curve (AUC), terminal half-life ($t_{1/2\gamma}$), apparent volumes of distribution, total plasma clearance (Cl_p), and micro-distribution rate constants. All calculations for pharmacokinetic parameters were based on methods described by Gibaldi and Perrier (Gibaldi & Perrier, 1982). All pharmacokinetic parameter estimates were calculated for each horse, and values are reported as median and range (minimum–maximum).

After intravenous administration of 1 mg of GLY, the observed plasma concentration vs. time profile in Standardbred horses was best described by a three-compartment model. The equation based on macroconstants for this model is as follows:

$$C_t = A_{\exp}{}^{-\alpha t} + B_{\exp}{}^{-\beta t} + C_{\exp}{}^{-\gamma t} \quad (1)$$

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

Renal and hepatic clearance

Renal clearance of GLY was determined using the following equation,

$$\frac{d_{Au}}{d_t} = Cl_R \cdot C_p \quad (2)$$

where d_{Au}/d_t is the rate of excretion of the drug into the urine, Cl_R is the renal clearance, and C_p is the plasma concentration. Hepatic clearance of GLY was estimated using the following equation (Wilkinson, 1987).

$$Cl_p = Cl_R + Cl_H + Cl_{other} \quad (3)$$

where Clp is the total plasma clearance, Cl_R is the renal clearance, Cl_H is the hepatic clearance, and Cl_{other} represents the sum of all other clearance processes.

Statistical analysis

Plots of urine and plasma concentrations and statistical analysis were performed using GraphPad Prism™ version 5.0 for Windows (GraphPad Software, San Diego, CA, USA), and plasma and urine concentrations of GLY are expressed as mean and standard deviation. Mann–Whitney U and Kruskal–Wallis rank-sum tests were used for statistical comparisons (nonparametric) of pharmacokinetic parameters between breeds (Hollander & Wolfe, 1973; Powers, 1990). Analysis of variance (ANOVA) was used for parametric analysis. A P-value of <0.05 was considered statistically significant. The 95/95% tolerance interval was calculated using JMP Version 9.0 (SAS Institute Inc., Cary, NC, USA).

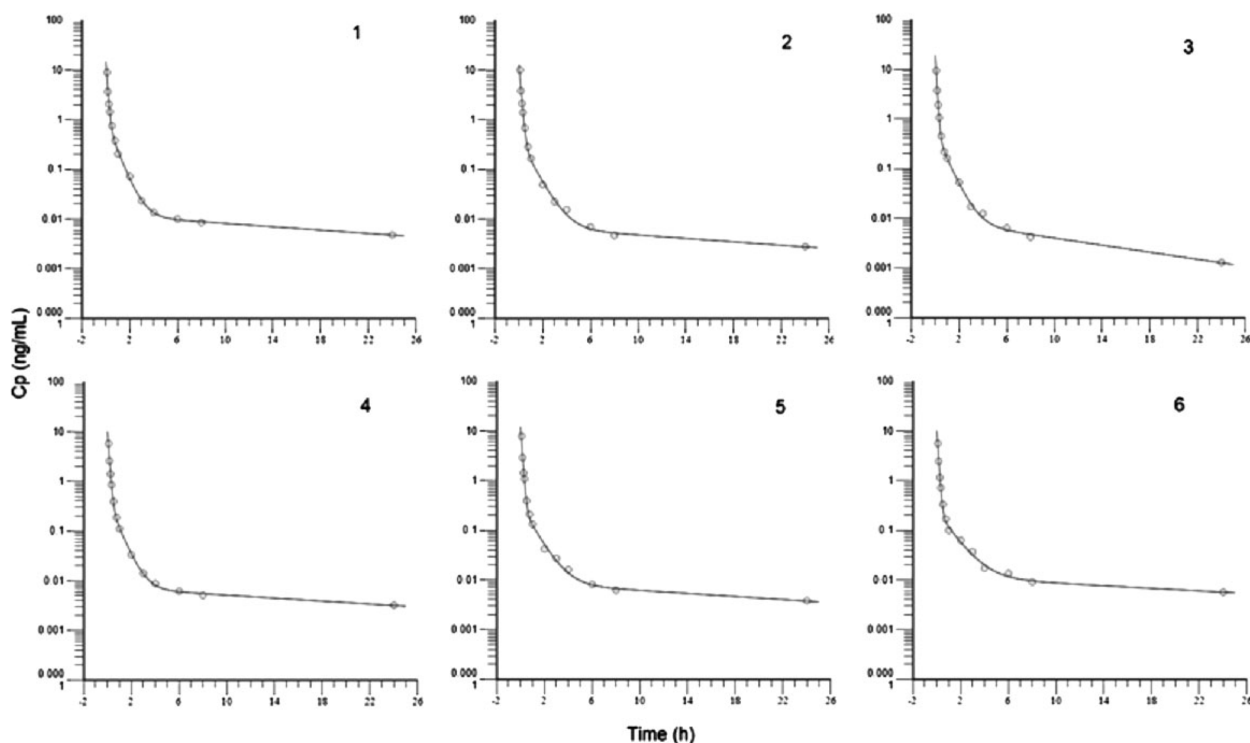

Fig. 1. Observed (open circles) and the predicted concentrations (line) vs. time when a three-compartment model is applied to six Standardbred horses after a single 1-mg IV injection of glycopyrrolate (GLY).

© 2013 John Wiley & Sons Ltd

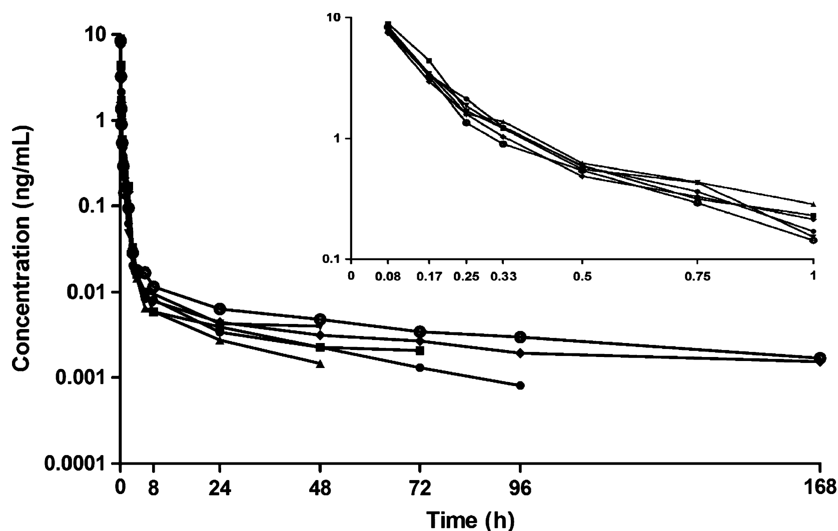

Fig. 2. Plasma concentration (ng/mL) vs. time (h) data from 0 to 168 h and 0 to 1 h (inset) for glycopyrrolate (GLY) administered intravenously to six healthy athletic adult Standardbred horses.

Table 1. Comparison of pharmacokinetics parameters of Standardbred (n = 6) and Thoroughbred horses (n = 6) following a 1-mg IV dose of glycopyrrolate (GLY)

	Standardbred			Thoroughbred*		
Parameter	Median	Min	Max	Median	Min	Max
A (ng/mL)	12.0	9.70	18.5	8.37	4.51	27.3
B (ng/mL)	0.410	0.229	0.912	0.404	0.076	2.23
C (ng/mL)	0.009	0.007	0.012	0.011	0.069	0.015
α (/h)	8.52	7.06	9.88	9.01	6.77	23.5
β (/h)	1.14	0.791	1.41	1.65	0.809	3.86
γ (/h)	0.037	0.030	0.082	0.094	0.056	0.119
C_{max} (ng/mL)	12.4	10.0	19.0	8.71	5.46	29.5
V_1 (L/kg)	0.170	0.103	0.215	0.212	0.065	0.336
K_{21} (/h)	1.36	0.975	1.85	1.93	0.884	4.88
K_{31} (/h)	0.041	0.038	0.086	0.102	0.062	0.125
K_{10} (/h)	5.90	5.51	7.92	6.56	5.58	17.1
K_{12} (/h)	1.28	0.904	1.63	1.44	0.505	4.32
K_{13} (/h)	0.817	0.368	1.50	0.651	0.494	1.23
K_{10}_HL (h)	0.117	0.088	0.126	0.106	0.041	0.124
$t_{1/2\alpha}$ (h)	0.081	0.070	0.098	0.077	0.030	0.102
$t_{1/2\beta}$ (h)	0.612	0.492	0.876	0.421	0.180	0.857
$t_{1/2\gamma}$ (h)	18.9	8.46	23.1	7.40	5.82	12.5
$AUC_{0\text{-inf}}$ (h*ng/mL)	2.58	2.28	2.88	1.52	1.22	2.41
Cl_p (mL/min/kg)	16.7	13.6	21.7	22.4	14.2	31.2
$AUMC_{0\text{-inf}}$ (h*h*ng/mL)	12.13	7.458	17.87	5.370	4.483	8.729
MRT (h)	3.04	1.73	4.28	1.43	0.449	4.64
V_{ss} (L/kg)	3.69	0.640	8.73	0.107	0.035	0.295
V_2 (L/kg)	0.146	0.094	0.317	1.15	0.349	4.08
V_3 (L/kg)	3.36	0.443	8.20	0.404	0.076	2.23

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, V_3, volumes of the central, second, and third compartments, respectively; K_{21}, K_{31}, K_{12}, 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. Min–minimum (n = 6); Max–maximum (n = 6); *Data taken from Rumpler *et al.* (2011b).

RESULTS

A three-compartment model describing the disposition of glycopyrrolate in plasma was chosen based on visual inspection of the observed and predicted concentration vs. time graphs for a two and three (Fig. 1) compartment model and AIC and SBC diagnostic criteria. Plasma GLY concentration vs. time plots for all six horses are depicted in Fig. 2. The GLY concentrations

© 2013 John Wiley & Sons Ltd

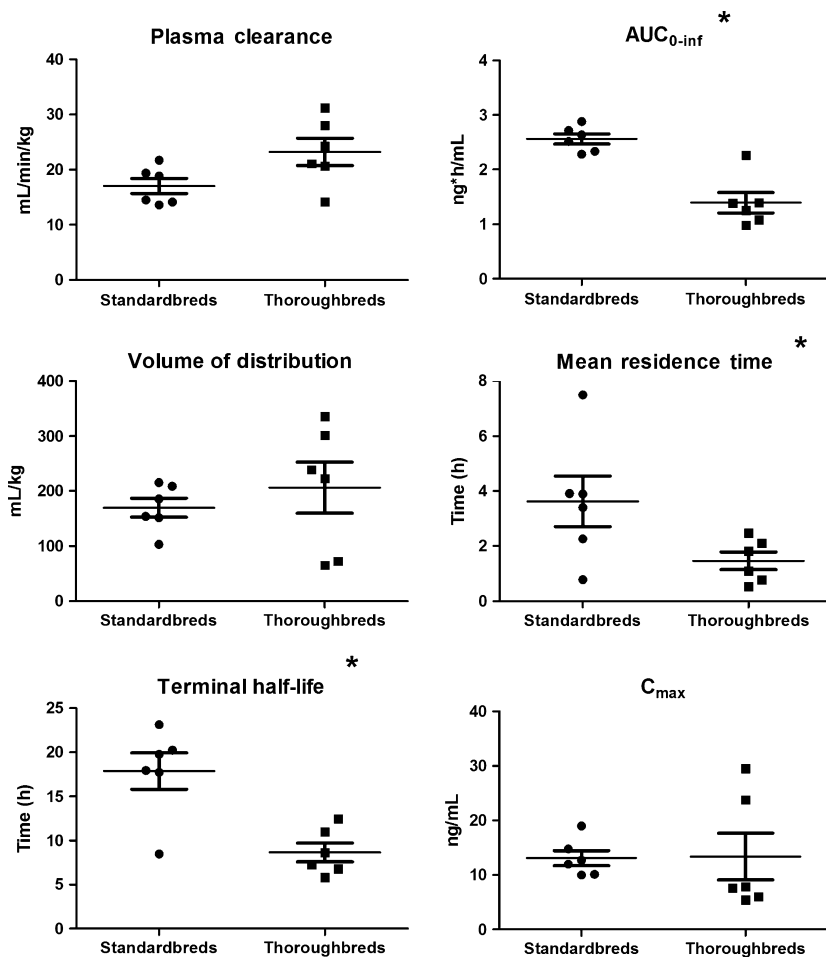

Fig. 3. Distribution of PK parameter estimates in Standardbred (n = 6) and Thoroughbred horses (n = 6). Calculated P-values using the Mann–Whitney U-test for comparisons of selected pharmacokinetic parameter estimates between breeds. * indicates statistical significance.

remained above the method's LLOQ (0.125 pg/mL) for all horses through 48 h and for two horses for 168 h after dosing. The upper limits of the 95/95 and 99/95 tolerance intervals for GLY in Standardbred horses at 48 h were 4.78 and 5.92 pg/mL, nearly three times greater than those in Thoroughbred horses (Rumpler *et al.*, 2011b).

The median (range) C_{max} in six Standardbred horses following a 1-mg intravenous dose of GLY was 12.4 (10.0–19.0) ng/mL. Median (range) estimates for plasma clearance (Cl_p), volume of distribution of the central compartment (V_1), volume of distribution at steady-state (Vss), and area under the plasma concentration–time curve ($AUC_{0\text{-inf}}$) were 16.7 (13.6–21.7) mL/min/kg, 0.167 (0.103–0.215) L/kg, 3.69 (0.640–38.73) L/kg, and 2.58 (2.28–2.88) ng*h/mL, respectively. Estimates for all of the pharmacokinetic parameters following compartmental model analysis for Standardbred and Thoroughbred horses (Rumpler *et al.*, 2011b) are reported in Table 1.

Figure 3 illustrates the distribution of six PK parameter estimates in six horses of each breed. Statistical comparisons were prepared using the Mann–Whitney U-test (Powers, 1990). The AUC0-inf, MRT, and terminal half-life of the Standardbred horses were different from those of Thoroughbred horses that were dosed similarly.

With regard to plasma esterase activity, the measured concentrations of GLY in fresh plasma incubated at 38 degrees for various times did not differ from the fortified concentrations by more than 10% and the GLY concentrations of all incubated samples differed from the zero-time sample by <5% indicating no appreciable hydrolysis. Average concentrations of GLY for duplicate analysis at the end of each incubation period through 360 min ranged from 9.05 to 10.3 pg/mL and were not different from the zero-time value.

For five of six horses, urine GLY concentrations peaked during the first collection interval. The total amount of GLY excreted in the urine through 24 h for six horses was characterized by a median (range) of 0.140 (0.113–0.246) mg or 14 (11.3–24.6)% of the total administered dose of 1 mg. Cumulative urinary excretion of GLY for each horse is shown in Fig. 4. Greater than 95% of the total renal excretion of GLY over 24 h occurred within the first 4 h after intravenous administration (Fig. 4). Urinary GLY excretion rates for each horse are shown in Fig. 4. Median (range) renal and nonrenal clearances for all six horses were estimated to be 2.65 (1.92–3.59) and 14.1 (11.7–18.1) mL/min/kg, respectively. Renal clearance represented approximately 11.3–24.7% of the total plasma clearance. Creatinine clearance ranged between 1.8 and 2.0 mL/min/kg.

© 2013 John Wiley & Sons Ltd

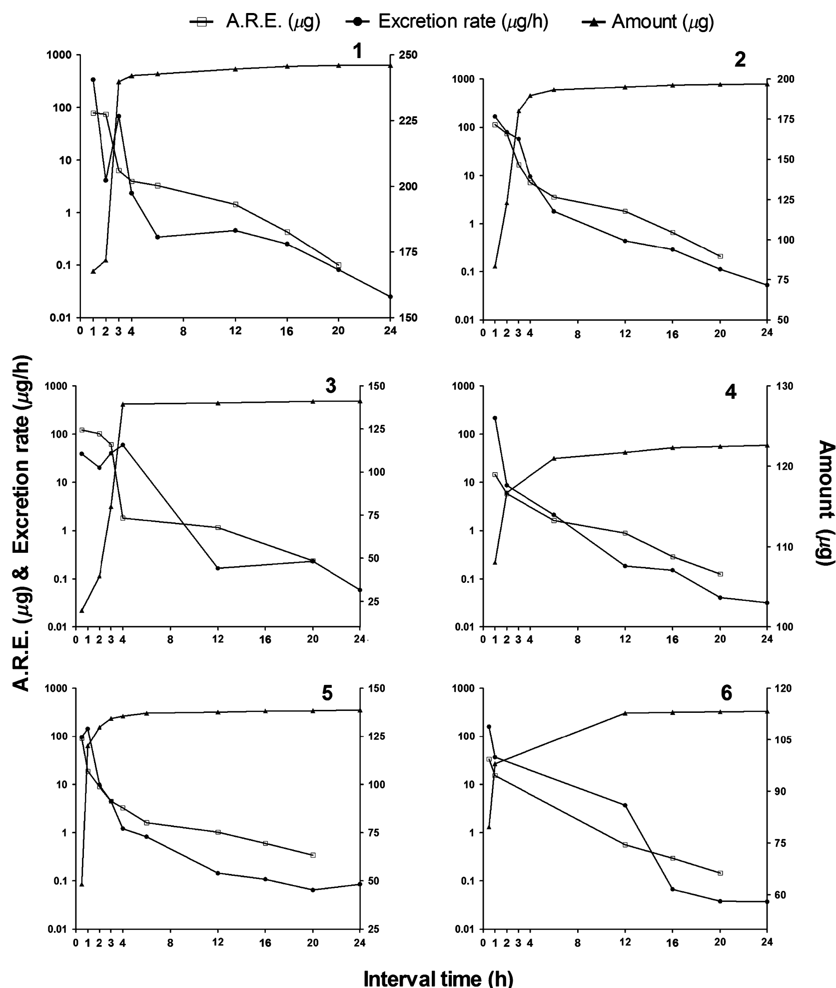

Fig. 4. Amount remaining to be excreted (A.R.E.) (μg), excretion rate (μg/h), and cumulative urinary excretion (μg) of unchanged glycopyrrolate (GLY) in six horses after a 1-mg intravenous injection. The A.R.E. and cumulative excretion are plotted against the end of the collection interval, while the excretion rate is plotted against the midpoint of the collection interval.

DISCUSSION

The pharmacokinetic profile of GLY after a single intravenous dose in Standardbred horses followed a tri-exponential decay, similar to its disposition in the Thoroughbred horse (Fig. 5). A three-compartment model compared to a two-compartment model provided a superior fit based on graphical inspection and lower diagnostic values for both AIC and SBC. GLY disposition was characterized by a rapid decline in plasma concentrations beginning immediately after administration, a comparatively small central volume of distribution but a large steady-state volume of distribution, and a slow terminal elimination attributed, in part, to the redistribution of GLY from the slow tissue compartment to the central compartment.

The cumulative excretion of unchanged GLY in horse urine indicated that <25% of the total dose was cleared by renal mechanisms. This observation contrasts with pharmacokinetic studies in humans that have reported that over 68% (Kaltiala *et al.*, 1974) and nearly 50% (Ali-melkkila *et al.*, 1990) of the intramuscularly administered dose was excreted unchanged in the urine. The latter study in humans reported that unchanged GLY was eliminated within three hours of administration and is consistent with the present study. The amount remaining to be excreted (ARE) plots demonstrate that, for this study, over 95% of the unchanged GLY was excreted within 4 h after drug administration. However, it is recognized that ARE calculations are subject to marginal error if complete urine collections are not prepared. As this study relied on the 'free-catch' method to collect the urine sample and did not use catheterization, collection volumes in a collection period may have been partial due to incomplete bladder emptying and the timing of the urine collections may have been less precise. Incomplete bladder emptying may result in an underestimation of the total amount of drug excreted during a collection interval but would not be expected to affect the total amount of drug excreted over the course of the study.

An alternative method for analyzing urine data to estimate half-life ($t_{1/2}$) and terminal rate constant (γ) is to plot the average rate of excretion against the time, which in most cases is best represented by the midpoint of the collection interval. The quality of the excretion rate plot has limitations due to incomplete bladder emptying and the need to collect accurately timed urine samples over short intervals relative to the elimination half-life of the drug (Gibaldi, 1986). However, an advantage of the rate of excretion plot is that each data point is essentially independent, especially if the bladder is fully

© 2013 John Wiley & Sons Ltd

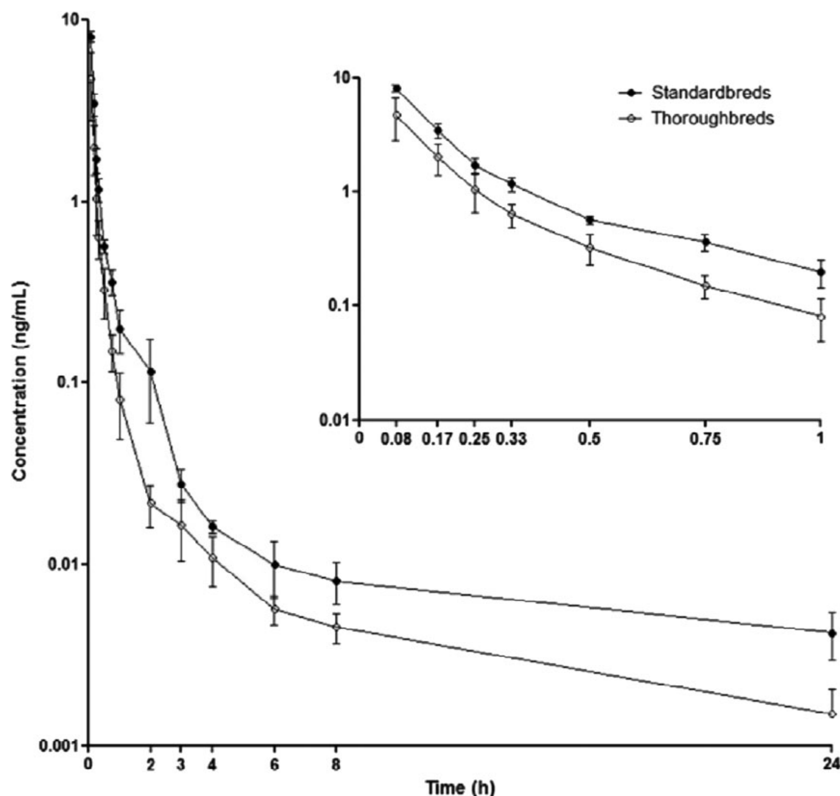

Fig. 5. Mean (SD) plasma concentration (ng/mL) vs. time (h) data from 0 to 24 h and 0 to 1 h for glycopyrrolate (GLY) administered intravenously to six Standardbred (●) and six Thoroughbred (○) horses (Rumpler *et al.*, 2011a).

voided for each sample. A missed sample or missed data points are not critical to this method. Moreover, the GLY excretion rate plots (Fig. 4) showed a large degree of scatter indicating uncertainty in this method of analysis probably due to the use of the free-catch method of urine collection and the comparatively rapid excretion compared with the length of the collection intervals.

Renal clearance of a substance can be determined by several methods, all of which are based on the relationship between the excretion rate and the corresponding plasma concentration as demonstrated in Equation 1-2. As shown in this equation, renal clearance is the constant of proportionality between the rate of excretion and the plasma concentration. Thus, a plot of the rate of excretion against the corresponding plasma concentration yields a straight line with a slope equal to renal clearance. Renal clearance was calculated using the rate of excretion method for the 0–24-h time interval.

The maximum rate at which GLY can be filtered by the glomerulus is the glomerular filtration rate (GFR) multiplied by the unbound fraction of GLY in plasma. The fraction of GLY bound to plasma protein over a range of plasma drug concentrations (0.1–25 ng/mL) is 37–44% (Rumpler *et al.*, 2013). Furthermore, estimates of the GFR can be obtained by measuring creatinine because it is filtered but neither secreted nor reabsorbed and calculating the creatinine renal clearance. We have determined that creatinine clearance ranged between 1.8 and 2.0 mL/min/kg in this study and was in agreement with estimates of creatinine clearance in mares (1.92 mL/min/kg) (Kohn & Strasser, 1986) and mature horses (1.67 mL/min/kg) (Zatzman *et al.*, 1982). Comparison of the renal clearance of GLY to the estimate of renal clearance from measurement of creatinine clearance multiplied by the free fraction indicated that secretion of GLY from the blood into the tubular lumen is likely because estimates for GLY renal clearance are higher than those of the free fraction times GFR (creatinine clearance). Reabsorption of unchanged GLY from the tubular lumen back into the blood via passive diffusion is unlikely due to the compound's polarity, although active reabsorption cannot be excluded. Therefore, these results suggest that GLY undergoes renal clearance through glomerular filtration of the free fraction and net tubular secretion.

Plasma esterase activity appeared to have little or no effect on the concentration of GLY, ruling out the contribution of plasma esterases to the total clearance (La du, 1972), such as the case with a number of other esters in the horse (Tobin *et al.*, 1976; Lehner *et al.*, 2000; Olsen *et al.*, 2007). A potential shortcoming of the current study is that plasma from only one horse was used to estimate esterase activity, and Kaminski *et al.* (1981) reported individual variability in esterase activity among horses. Despite this and in light of the urinary GLY clearance data, it can be hypothesized that the majority of nonrenal clearance is hepatic clearance, and further, the majority of total clearance is attributed to hepatic clearance Equation 1-3. Hepatic clearance has been estimated in this study as the difference between Cl_R and Clp, assuming that Cl_{other} is relatively small. Total plasma clearance in Standardbred horses was characterized by a median (range) of 16.7 (13.6–21.7) mL/min/kg. Therefore, using the renal clearance of 2.65 (1.92–3.59) mL/min/kg, nonrenal clearance was approximately 14.1 (11.7–18.1) mL/min/kg.

© 2013 John Wiley & Sons Ltd

The median (range) estimates of hepatic clearance in both Thoroughbred (20.1 (12.3–27.6) mL/min/kg) and Standardbred horses (14.1 (11.7–18.1) mL/min/kg) closely approximate previous estimates of liver blood flow in the horse (15–20 mL/min/kg) (Dyke *et al.*, 1998). Under such conditions whereby the plasma clearance is equivalent to the hepatic blood flow, the drug is said to be highly extracted by the liver (Wilkinson & Shand, 1975).

$$E_H = \frac{\mathrm{Cl_H}}{Q} \quad (4)$$

Where $\mathrm{Cl_H}$ is the hepatic blood clearance, Q is the liver blood flow, and $\mathrm{E_H}$ is the hepatic extraction ratio, a term representing the fraction of dose undergoing metabolism and thus irreversible removal. In addition, for drugs with a high extraction ratio, changes in liver blood flow are a major determinant of hepatic clearance and the degree of, or changes in, the protein binding of GLY have little influence on hepatic clearance (Sansom & Evans, 1995). In the horse, GLY is extensively metabolized in the liver by hydrolysis of the ester moiety to 2-cyclopentyl-2-hydroxy-2-phenylacetic acid (cyclopentylmandelic acid) and 1,1-dimethyl-3-hydroxypyrrolidine-1-ium.

Although disposition of GLY was similar in Thoroughbred and Standardbred horses following intravenous administration, differences in some pharmacokinetic parameter estimates were evident. The AUC values were greater in Standardbred horses than in Thoroughbred horses, but the total plasma clearances were not different (16.7 (13.6–21.7) mL/min/kg in Standardbred horses compared with 22.4 (14.2–31.2) mL/min/kg in Thoroughbred horses). There are no known reports detailing differences in hepatic blood flow between breeds which would account for the small but insignificant difference in total plasma clearance between breeds. Additionally, while differences in drug distribution between donkeys and horses have been reported for phenylbutazone (Mealey *et al.*, 1997), guaifenesin (Matthews *et al.*, 1997), and sulfamethoxazole (Peck *et al.*, 2002), there is little information detailing pharmacokinetic or differences in drug metabolism, if any, between Standardbred and Thoroughbred horse breeds. Despite there being no known reports of differences between hepatic blood flow or cardiac output between Standardbred and Thoroughbred horses, Hackett *et al.* (2003) reported that heart rates do not differ between Thoroughbred and Standardbred horses at rest. After exercise, however, Standardbred horses exhibited faster heart rates at similar fractions of maximal heart rate compared with Thoroughbred horses. Heart rate was not measured in either of our studies, so it is unknown whether there was a breed difference and whether this may have caused an increase in hepatic blood flow via increased cardiac output.

CONCLUSION

We have demonstrated the disposition of GLY in Standardbred horses and, with our previous work in Thoroughbred horses, presented one of the few comparisons between pharmacokinetic parameters for any drug between two performance horse breeds. We have also provided estimates of GLY renal clearance in order to understand its contribution to total GLY clearance. We conclude that the majority of GLY is cleared through hepatic mechanisms because of the limited degree of GLY renal clearance and absence of hydrolysis in plasma as a result of plasma esterase activity.

ACKNOWLEDGMENTS

The authors wish to thank the Racing Medication and Testing Consortium, Inc (Lexington, KY) and the Florida Division of Pari-Mutuel Wagering (Tallahassee, FL) for financial support for this work. We also thank Brett Rice of Large Animal Clinical Sciences at the University of Florida for technical advice and performing the treadmill exercise studies and sample collections.

REFERENCES

Akaike, H. (1976) An information criterion (AIC). *Mathematical Sciences*, **14**, 5–9.

Ali-melkkila, T.M., Kaila, T., Kanto, J. & IIsalo, E. (1990) Pharmacokinetics of I.M. glycopyrronium. *British Journal of Anaesthesia*, **64**, 667–669.

Chen, J. & Hsieh, Y. (2005) Stabilizing drug molecules in biological samples. *Therapeutic Drug Monitoring*, **27**, 617–624.

Dyke, T.M., Hubbell, J.A., Sams, R.A. & Hinchcliff, K.W. (1998) Hepatic blood flow in horses during the recuperative period from maximal exercise. *American Journal of Veterinary Research*, **59**, 1476–1480.

Gabrielsson, J. & Weiner, D. (2007) *Pharmacokinetic and Pharmacodynamic Data Analysis, Concepts and Applications*. Swedish Pharmaceutical Press, Stockholm, Sweden.

Gibaldi, M. (1986) The basic concept: clearance. *Journal of Clinical Pharmacology*, **26**, 330–331.

Gibaldi, M. & Perrier, D. (1982) *Pharmacokinetics*. Marcel Decker, New York, NY.

Hackett, R.P., Ducharme, N.G., Gleed, R.D., Mitchell, L., Soderholm, B.K. & Erickson, H.N. (2003) Do thoroughbred and standardbred horses ave similar increases in pulmonary vascular pressures during exertion. *The Canadian Journal of Veterinary Research*, **67**, 291–296.

Hervey, G.R. (1953) Determination of creatinine by the Jaffé reaction. *Nature*, **20**, 1125.

Hollander, M. & Wolfe, D.A. (1973) *Nonparametric Statistical Methods*. John Wiley and Sons, New York.

Kaltiala, E., Penttila, A., Vapaatalo, H. & Larmi, T. (1974) The fate of intravenous (3H) glycopyrrolate in man. *Journal of Pharmacy and Pharmacology*, **26**, 352–354.

Kaminski, M., Sykiotis, M. & Didkowski, S. (1981) Horse serum esterase: investigations on the relationship between qualitative and quantitative expression of allelic genes. *Biochemical Systematics and Ecology*, **9**, 355–364.

Kohn, C.W. & Strasser, S.L. (1986) 24-Hour renal clearance and excretion of endogenous substances in the mare. *American Journal of Veterinary Research*, **47**, 1332–1337.

La du, B. (1972) Plasma esterase activity and the metabolism of drugs with ester compounds. *Annals of the New York Academy of Sciences*, **179**, 684–693.

© 2013 John Wiley & Sons Ltd

Lehner, A.F., Almeida, P., Jacobs, J., Harkins, J.D., Karpiesiuk, W., Woods, W.E., Dirikolu, L., Bosken, J.M., Carter, W.G., Boyles, J., Holtz, C., Heller, T., Nattrass, C., Fisher, M. & Tobin, T. (2000) Remifentanil in the horse: identification and detection of its major urinary metabolite. *Journal of Analytical Toxicology*, **24**, 309–315.

Matthews, N.S., Peck, K.E., Mealey, K.L., Taylor, T.S. & Ray, A.C. (1997) Pharmacokinetics and cardiopulmonary effects of guaifenesin in donkeys. *Journal of Veterinary Pharmacology and Therapeutics*, **20**, 442–446.

Mealey, K.L., Matthews, N.S., Peck, K.E., Ray, A.C. & Taylor, T.S. (1997) Comparative pharmacokinetics of phenylbutazone and its metabolite oxyphenbutazone in clinically normal horses and donkeys. *American Journal of Veterinary Research*, **58**, 53–55.

Mirakhur, R.K. (1985) Glycopyrrolate and human plasma cholinesterase. *Canadian Anaesthetists' Society Journal*, **32**, 683–684.

Olsen, L., Ingvast-larsson, C., Brostrom, H. & Tjalve, H. (2007) Clinical signs and etiology of adverse reactions to procaine benzylpenicillin and sodium/potassium benzylpenicillin in horses. *Journal of Veterinary Pharmacology and Therapeutics*, **30**, 201–207.

Peck, K.E., Matthews, N.S., Taylor, T.S. & Mealey, K.L. (2002) Pharmacokinetics of sulfamethoxazole and trimethoprim in donkeys, mules, and horses. *American Journal of Veterinary Research*, **63**, 349–353.

Powers, J.D. (1990) Statistical analysis of pharmacokinetic data. *Journal of veterinary pharmacology and therapeutics*, **13**, 113–120.

Rowland, M. & Tozer, T.N. (1995) *Clinical Pharmacokinetics, Concepts and Applications*. Williams and Wilkins, Baltimore.

Rumpler, M.J., Sams, R.A. & Colahan, P. (2011a) Validation of an LC/MS/MS method for quantification of glycopyrrolate in horse plasma. *Journal of Analytical Toxicology*, **35**, 656–664.

Rumpler, M.J., Sams, R.A. & Colahan, P. (2011b) Pharmacokinetics of glycopyrrolate following intravenous administration in the horse. *Journal of Veterinary Pharmacology and Therapeutics*, **34**, 605–608.

Rumpler, M.J., Sams, R.A. & Colahan, P. (2012) Regulatory control of glycopyrrolate in the horse. *Journal of Chromatography B*, **889–890**, 130–137.

Rumpler, M.J., Kandala, B., Vickroy, T., Hocchaus, G. & Sams, R.A. (2013) Pharmacokinetics and pharmacodynamics of glycopyrrolate following a continuous-rate infusion in the horse. *Journal of Veterinary Pharmacology and Therapeutics*, doi: 10.1111/jvp.12074. [Epub ahead of print].

Sansom, L.N. & Evans, A.M. (1995) What is the true clinical significance of plasma protein binding displacement interactions? *Drug Safety*, **12**, 227–233.

Satoh, T., Taylor, P., Bosron, W.F., Sanghani, S.P., Hosokawa, M. & La Du, B.N. (2002) Current progress on esterases: from molecular structure to function. *Drug Metabolism and Disposition*, **30**, 488–493.

Schwarz, G.E. (1978) Estimating the dimension of a model. *Annals of Statistics*, **6**, 461–464.

Tobin, T., Blake, J.W., Sturma, L. & Arnett, S. (1976) Pharmacology of procaine in the horse: procaine esterase properties of equine plasma and synovial fluid. *American Journal of Veterinary Research*, **37**, 1165–1170.

Wilkinson, G.R. (1987) Clearance approaches in pharmacology. *Pharmacological Reviews*, **39**, 1–47.

Wilkinson, G.R. & Shand, D.G. (1975) Commentary: a physiological approach to hepatic drug clearance. *Clinical Pharmacology and Therapeutics*, **18**, 377–390.

Yamaoka, K., Nakagawa, T. & Uno, T. (1978) Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. *Journal of Pharmacokinetics and Biopharmaceutics*, **6**, 165–175.

Zatzman, M.L., Clarke, B.A., Ray, W.J., Garner, H.E. & Traver, D. (1982) Renal function of the pony and the horse. *American Journal of Veterinary Research*, **43**, 608–612.

Zsigmond, E.K., Winnie, A.P., Barabas, E. & Wang, X.Y. (1985) The inhibitory effect of glycopyrrolate on human plasma cholinesterase. *Canadian Anaesthetists' Society Journal*, **32**, 20–22.

© 2013 John Wiley & Sons Ltd