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Pharmacokinetics of glycopyrrolate following intravenous administration in the horse

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Glycopyrrolate, designated a class 3 substance by the Association of Racing Commissioners International, Inc., is regulated in racing horses because of its potential to affect performance. Although it has veterinary clinical applications by inhibiting parasympathetic activity, its use near race day is prohibited and positive reports from postrace samples in the US are relatively common. Accordingly, the American Association of Equine Practitioners identified glycopyrrolate as a therapeutic substance used by race track practitioners for legitimate therapeutic purposes, and the Racing Medication and Testing Consortium (RMTC) has requested studies of the disposition of glycopyrrolate as part of its efforts to acquire reliable data upon which to propose thresholds and withdrawal time recommendations for therapeutic substances used in racing horses.

Glycopyrrolate, a quaternary ammonium salt and synthetic anti-cholinergic drug, exerts peripheral anti-muscarinic effects on the respiratory tract without imparting substantial effects on the central nervous system (CNS) compared to other muscarinic antagonists such as atropine. Glycopyrrolate differs from these other muscarinic antagonists because it penetrates the CNS poorly due to its highly polar quaternary ammonium group and its permanent ionization at physiological pH compared to its more lipophilic congeners.

Previous studies have investigated glycopyrrolate pharmacokinetics in humans to a limited extent (Pentilla *et al.*, 2001). However, to our knowledge, pharmacokinetic studies of this drug in the horse have not been reported likely due to limitations in sensitivity of the methods that are commonly used. Quantitative methods with limits of detection and quantification well below those of previously reported methods have recently been developed and validated through the RMTC research program. These validated methods (Rumpler *et al.*, 2010b) demonstrate necessary sensitivity, accuracy, and precision to measure plasma concentrations sufficient to perform pharmacokinetic analysis through the 24-h time period after administration of clinically relevant doses to horses. Such investigations could contribute to the RMTC effort to establish a plasma threshold and to recommend a withdrawal time for this drug in race horses. Therefore, this study investigated the disposition of glycopyrrolate following intravenous administration of a 1-mg dose in the horse.

Eight, healthy, adult, Thoroughbred geldings, ranging in age from 5 to 10 years and weighing from 518 to 580 kg were used in these studies. All study horses were housed in grass paddocks at the University of Florida, Veterinary Medical Center (Gainesville, FL), maintained on a diet of commercially available grain mixture, and had open access to water and hay at all times. Horses were subjected to treadmill exercise (3 days/week) before and throughout the duration of these studies. The experimental protocol was approved, and facilities were inspected by the University of Florida Institutional Animal Care and Use Committee.

All horses were administered 1 mg (1.72–1.93 μg/kg) of glycopyrrolate (glycopyronium bromide, American Regent, Inc., Shirley, NY, USA) into the right jugular vein. Whole blood samples were collected from the left jugular vein via needle venipuncture into partially evacuated tubes containing lithium heparin. Blood samples were stored on ice until the plasma was concentrated by centrifugation (2500–3000 rpm or 776–1318 ***g***) at 4 °C for 15 min. Harvesting of plasma took place within 1 h of sample collection, and 2–4-mL aliquots of plasma were immediately frozen at −20 °C and stored within 24 h at −80 °C until analyzed. Collection times included a timepoint before drug administration and 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. Specimens were collected from two of the horses only through 24 h after dosing.

Plasma glycopyrrolate concentrations were determined using a fully validated ultra-performance liquid chromatography and tandem mass spectrometry (MS/MS) method as previously described (Rumpler *et al.*, 2010b) in accordance with US FDA recommended guidelines for bioanalytical methods. The method is characterized by a lower limit of quantitation (LLOQ) of 0.05 pg/mL of plasma.

Nonlinear least squares regression analysis was performed on plasma glycopyrrolate concentration vs. time data and pharmacokinetic parameters for all horses were estimated with both noncompartmental and compartmental analysis using Phoenix

WinNonlin® 6.1 (Pharsight, St. Louis, MO, USA). For compartmental analysis, 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 (Yamaoka *et al.*, 1978) and Schwartz's Bayesian Criterion 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 microdistribution rate constants. For the noncompartmental analysis, the area under the plasma concentration vs. time curve (AUC_{0-24}) from time 0 to 24 h was calculated using the log-linear trapezoidal method with linear interpolation. The pharmacokinetic parameters calculated included the observed maximum plasma concentration (C_{max}), area under the plasma concentration vs. time curve to the last determined plasma concentration (AUC_t), terminal half-life ($t_{1/2}$), total plasma clearance (Cl_p), mean residence time, and steady state volume of distribution (V_{ss}). All calculations for pharmacokinetic parameters were based on methods described by Gibaldi and Perrier (1982). All pharmacokinetic parameters were calculated for each horse, and values are reported as median and range (minimum-maximum).

After intravenous administration of 1 mg of glycopyrrolate, the observed plasma concentration vs. time profile could be best described by a three-compartment model. The equation based on macro constants for this model is:

$$C_t = \mathrm{A}\exp^{-\alpha\cdot t} + \mathrm{B}\exp^{-\beta\cdot t} + \mathrm{C}\exp^{-\gamma\cdot t}$$

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. Values for a number of pharmacokinetic variables following noncompartmental and compartmental model analysis are reported in Tables 1 and 2, respectively. Plasma glycopyrrolate concentration vs. time plots for all eight horses are depicted in Fig. 1. The drug concentrations remained above 0.5 pg/mL for all horses through 24 h after dosing (Fig 1).

To our knowledge, the pharmacokinetics of glycopyrrolate in the horse have not previously been investigated. Our data indicate that glycopyrrolate disposition in the horse exhibits triexponential decay after intravenous administration. This is characterized by an early rapid decline (Fig. 1) in plasma concentrations followed by a slow terminal phase with concentrations above the LOQ of the method for up to 168 h. All horses exhibited plasma concentrations above 1 ng/mL 5 min after drug administration followed by a precipitous decline through 20 min. Although the three-compartment model estimates for C_{max} are higher than the noncompartmental estimates because of the extrapolation back to time 0 in the compartmental model, we believe that the inclusion of these values in the model is necessary to describe the disposition of glycopyrrolate (Beaufort *et al.*, 1999). Moreover, data in humans suggest a similar pharmacokinetic profile (Pentilla *et al.*, 2001). Noncompartmental analysis provided physiologically reasonable parameter estimates. However, the volume of distribution based on the terminal phase (V_z) was unrealistically large (16.9 ± 6.7 L/kg), likely accounted for by the rapid elimination during the initial phase and low plasma glycopyrrolate concentrations during the terminal phase (Toutain & Bousquet-Melou, 2004).

Total plasma clearance is attributed to hydrolysis of glycopyrrolate and renal clearance (Rumpler *et al.*, 2010a). Although our previous studies have revealed that some glycopyrrolate is eliminated unchanged in the urine, we did not perform volumetric urine collections in this study and therefore cannot

Table 1. Pharmacokinetic parameter estimates of glycopyrrolate, determined using noncompartmental analysis, following intravenous administration of 1 mg to eight (n = 8) healthy adult Thoroughbred horses

	Horse										
Parameter	1	2	3	4	5	6	7	8	Median	Min	Max
λ_z (h^{-1})	0.097	0.066	0.089	0.102	0.084	0.082	0.067	0.054	0.083	0.066	0.102
$t_{1/2}\lambda_z$ (h)	7.14	10.5	7.79	6.78	8.28	8.48	10.4	12.9	8.38	6.78	12.9
C_{max} (ng/mL)	5.48	4.72	4.21	8.27	5.14	4.07	2.43	4.55	4.64	2.43	8.27
C_{last} (ng/mL) × 10^{-3}	1.11	1.54	1.17	0.860	1.25	0.953	2.25	1.92	1.21	0.860	2.25
AUC_{0-24} (h*ng/mL)	1.67	1.40	1.38	2.49	1.54	1.43	0.953	1.50	1.46	0.953	2.49
$AUC_{0-\infty}$ (h*ng/mL)	1.68	1.42	1.40	2.50	1.55	1.44	0.987	1.53	1.49	0.987	2.50
V_z (L/kg)	12.5	19.4	14.1	7.56	13.1	14.6	27.9	22.2	14.4	7.56	27.9
Cl (mL/min/kg)	20.3	21.3	21.0	12.9	18.3	19.9	31.0	19.9	20.1	12.9	31.0
$AUMC_{0-24}$ (h*h*ng/mL)	1.06	0.963	1.04	0.952	0.990	0.783	1.40	1.20	1.01	0.783	1.40
MRT_{0-24} (h)	0.636	0.688	0.750	0.383	0.644	0.548	1.47	0.798	0.666	0.383	1.47
V_{ss} (L/kg)	1.05	1.68	1.35	0.383	1.08	1.00	5.13	2.10	1.22	0.383	5.12

λ_z, elimination rate constant; $t_{1/2}\lambda_z$, terminal half-life; C_{max}, observed maximum plasma glycopyrrolate concentration; C_{last}, observed plasma glycopyrrolate concentration at 24 h; AUC_{0-24}, area under the plasma concentration vs. time curve from time 0 to 24 h; V_z, volume of distribution based on the terminal phase; Cl_t, observed total plasma clearance; $AUMC_{0-24}$, area under the first moment curve from time 0 to 24 h; MRT_{0-24}, mean residence time from time 0 to 24 h; V_{ss}, volume of distribution at steady state.

Table 2. Pharmacokinetic parameter estimates of glycopyrrolate, determined using a three-compartmental model, following intravenous administration of 1 mg to eight (n = 8) healthy adult Thoroughbred horses

	Horse										
Parameter	1	2	3	4	5	6	7	8	Median	Min	Max
A (ng/mL)	9.72	5.96	7.49	27.3	9.24	22.8	4.51	7.32	8.37	4.51	27.3
B (ng/mL)	0.436	0.076	0.371	2.23	0.281	0.954	0.935	0.331	0.404	0.076	2.23
C (ng/mL)	0.012	0.069	0.011	0.014	0.011	0.098	0.015	0.071	0.011	0.069	0.015
Alpha (h^{-1})	9.16	6.77	7.30	17.3	8.73	23.5	10.8	8.86	9.01	6.77	23.5
Beta (h^{-1})	1.73	0.809	1.53	3.86	1.57	2.95	2.45	1.09	1.65	0.809	3.86
Gamma (h^{-1})	0.101	0.063	0.095	0.119	0.092	0.102	0.080	0.056	0.094	0.056	0.119
C_{max} (ng/mL)	10.2	6.04	7.88	29.5	9.54	23.8	5.46	7.66	8.71	5.46	29.5
V_1 (L/kg)	0.201	0.302	0.223	0.065	0.179	0.073	0.336	0.239	0.212	0.065	0.336
K_{21} (h^{-1})	2.05	0.884	1.80	4.88	1.79	3.78	3.89	1.43	1.93	0.884	4.88
K_{31} (h^{-1})	0.110	0.070	0.104	0.125	0.101	0.110	0.098	0.062	0.102	0.062	0.125
K_{10} (h^{-1})	7.08	5.58	5.69	13.0	7.03	17.1	5.58	6.10	6.56	5.58	17.1
K_{12} (h^{-1})	1.12	0.505	0.838	2.60	0.830	4.32	2.58	1.76	1.44	0.505	4.32
K_{13} (h^{-1})	0.628	0.603	0.494	0.668	0.646	1.23	1.19	0.656	0.651	0.494	1.23
K_{10}_HL (h)	0.098	0.124	0.122	0.053	0.099	0.041	0.124	0.114	0.106	0.041	0.124
$t_{1/2\alpha}$ (h)	0.076	0.102	0.095	0.040	0.079	0.030	0.064	0.078	0.077	0.030	0.102
$t_{1/2\beta}$ (h)	0.401	0.857	0.454	0.180	0.441	0.235	0.283	0.635	0.421	0.180	0.857
$t_{1/2\gamma}$ (h)	6.89	11.0	7.28	5.82	7.52	6.77	8.61	12.5	7.40	5.82	12.5
AUC_{0-24} (h*ng/mL)	1.43	1.08	1.39	2.27	1.36	1.39	0.979	1.26	1.37	0.979	2.27
Cl_t (mL/min/kg)	23.8	28.0	21.1	14.2	20.9	20.7	31.2	24.3	22.4	14.2	31.2
$AUMC_{0-24}$ (h*h*ng/mL)	1.47	1.98	1.52	1.20	1.52	1.09	2.43	2.65	1.51	1.09	2.65
V_{ss} (L/kg)	1.46	3.07	1.39	0.449	1.41	0.967	4.64	3.08	1.43	0.449	4.64
V_2 (L/kg)	0.110	0.172	0.104	0.035	0.083	0.083	0.222	0.295	0.107	0.035	0.295
V_3 (L/kg)	1.15	2.60	1.06	0.349	1.14	0.812	4.08	2.54	1.15	0.349	4.08

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 glycopyrrolate 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; $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$, rapid elimination half-life; $t_{1/2\gamma}$, slow elimination half-life; AUC, area under the plasma concentration vs. time curve; Cl_t, total plasma clearance; AUMC, area under the first moment curve; V_{ss}, volume of distribution at steady state.

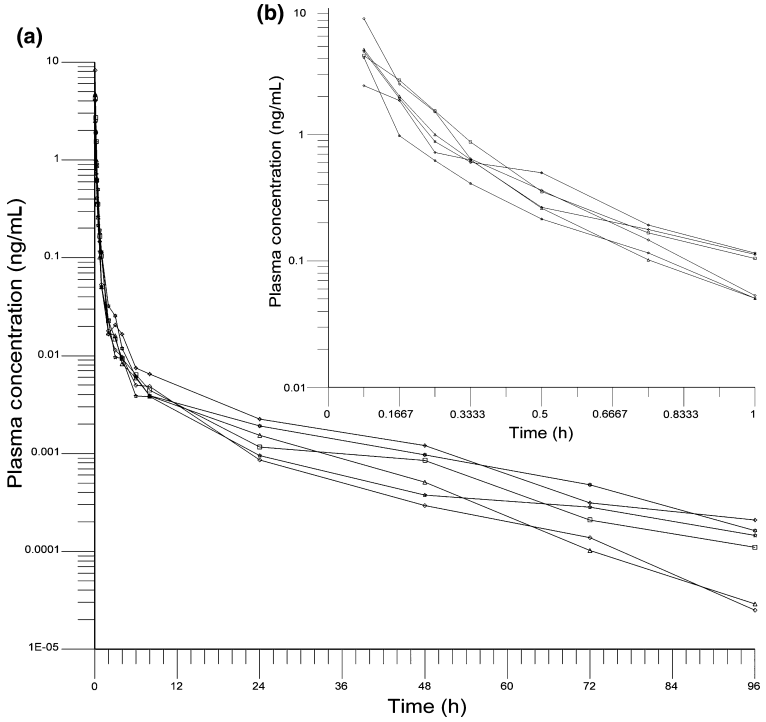

Fig. 1. Plasma concentration (ng/mL) vs. time (h) data from (a) 0–96 h and (b) 0–1 h for glycopyrrolate administered intravenously in eight healthy adult Thoroughbreds.

estimate renal clearance of glycopyrrolate. The total plasma clearance of glycopyrrolate from this study is approximately equal to previous estimates of hepatic blood flow in the horse (Dyke *et al.*, 1998), suggesting that renal clearance may be relatively small. Furthermore, studies in humans, following a single intravenous dose, estimate plasma clearance values to be 16.8 ± 3.83 (mean ± SD) and 18.1 (10–23.8) (median and range) mL/min/kg (Rautakorpi *et al.*, 1998 and Pentilla *et al.*, 2001), closely approximating human hepatic blood flow (Davies *et al.*, 1993).

In conclusion, plasma pharmacokinetics of glycopyrrolate in the horse following a single intravenous clinically relevant dose can be characterized by a three-compartment model. A wide distribution from the central compartment, rapid clearance, and prolonged terminal half-life were observed. Further studies are needed to determine the extent of the contribution of renal clearance and plasma hydrolysis to the total plasma clearance. We believe the current study contributes reliable data upon which to recommend a withdrawal time and threshold limit for the therapeutic use of glycopyrrolate in racing horses.

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