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Pharmacokinetics of guaifenesin following administration of multiple doses to exercised Thoroughbred horses

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Guaifenesin is an expectorant commonly used in performance horses to aid in the clearance of mucus from the airways. Guaifenesin is also a centrally acting skeletal muscle relaxant and as such is a prohibited drug with withdrawal necessary prior to competition. To the authors' knowledge, there are no reports in the literature describing single or multiple oral administrations of guaifenesin in the horse to determine a regulatory threshold and related withdrawal time. Therefore, the objective of the current study was to describe the pharmacokinetics of guaifenesin following oral administration in order to provide data upon which appropriate regulatory recommendations can be established. Nine exercised Thoroughbred horses were administered 2 g of guaifenesin orally BID for a total of five doses. Blood samples were collected immediately prior to drug administration and at various times postadministration. Serum guaifenesin concentrations were determined and pharmacokinetic parameters calculated. Guaifenesin was rapidly absorbed (T_{max} of 15 min) following oral administration. The C_{max} was 681.3 ± 323.8 ng/mL and 1080 ± 732.8 following the first and last dose, respectively. The serum elimination half-life was 2.62 ± 1.24 h. Average serum guaifenesin concentrations remained above the LOQ of the assay (0.5 ng/mL) by 48 h postadministration of the final dose in 3 of 9 horses.

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Guaifenesin is a centrally acting skeletal muscle relaxant and as such is used commonly in veterinary medicine as an adjunct in anesthetic protocols. A more common use for guaifenesin in human medicine is as an expectorant. Irritation of the gastric mucosa by guaifenesin stimulates respiratory tract secretions, thereby increasing respiratory fluid volumes and decreasing mucus viscosity (Kagan *et al.*, 2009). Although its efficacy as an expectorant in horses has not been well studied and its efficacy appears to be anecdotal, the use of guaifenesin in horses as an expectorant, especially performance horses is common.

To the authors' knowledge, there are a limited number of reports describing drug concentrations and the pharmacokinetics of guaifenesin in the horse (Davis & Wolf, 1970; Hubbell *et al.*, 1980; Matthews *et al.*, 1997). Furthermore, there are no reports in the literature describing oral or multiple administrations of guaifenesin in the horse. Therefore, the objective of the current study was to describe serum concentrations and the pharmacokinetics of guaifenesin following multiple oral administrations to horses, utilizing a highly sensitive liquid chromatography–mass spectrometry (LC-MS/MS) method currently employed by drug testing laboratories to analyze biological samples collected from racehorses. The use of guaifenesin in racehorses limited pharmacokinetic data and its classification as a class 4 foreign substance (a skeletal muscle relaxant without prominent central nervous system effects) by the Association of Racing Commissioners International (ARCI) warrant further study of this drug to establish appropriate regulatory recommendations.

Nine healthy exercised adult Thoroughbred horses including seven geldings and two mares (4–9 years of age; weight of 550–563 kg) were studied. Prior to the study, horses were exercised 6 days a week including 3 days on an Equigym® treadmill (Equigym, LLC, Lexington, KY, USA) and 3 days on a mechanical walker (Equigym, LLC). Before beginning the study, horses were weighed and determined healthy and free of

disease by physical examination, complete blood count, and a serum biochemistry panel. Horses did not receive any other medications for at least 2 weeks prior to commencement of this study. This study was approved by the Institutional Animal Care and Use Committee of Kentucky Equine Research (KER, University of Kentucky, and Rood and Riddle Equine Hospital).

Prior to drug administration, a 14-gauge catheter was aseptically placed in one external jugular vein for sample collection. Guaifenesin powder (Spec-tuss; Neogen Corporation, Lexington, KY, USA) was administered suspended in water (60 mL) via a dosing syringe at a dose of 2 g (3.5–3.6 mg/kg) BID for a total of five doses.

Blood samples were collected at time 0, 15, and 30 min and 1, 2, 4, 6, and 12 h after the first dose, at 0, 15, and 30 min and 1, 2, 4, 6, 12, 24, 36, and 48 h after the last dose. Additional samples were collected at 12-h intervals during the dosing period (immediately prior to drug administration). Prior to drawing each sample of blood, 10 mL of blood was aspirated from the catheter and T-port extension set and discarded. The catheter was flushed with 10 mL of a dilute heparinized saline solution (10 IU/mL) following each sampling. Blood samples (15 mL) were collected into serum separator tubes and placed on ice prior to centrifugation at 3000 ***g*** for 10 min. Serum was transferred into storage cryovials and stored at −20 °C until shipped to the laboratory for analysis. Samples were shipped on dry ice and immediately transferred to a −20 °C freezer upon receipt.

Prior to analysis, 1 mL of serum was diluted with 0.2 mL of water containing 200 ng/mL of guaifenesin-d_5 internal standard (Toronto Research Chemicals, Toronto, ON, Canada) and 4 mL of MTBE: methylene chloride (60:40 v:v) was added to each serum sample, and the samples were mixed by rotation for 20 min at 40 revolutions per minute followed by centrifugation at 2260 ***g*** for 5 min at 4 °C. The top organic layer was transferred to a 12 × 75 mm glass tube, dried under nitrogen, and dissolved in 100 μL of 5% acetonitrile in water. The injection volume was 40 μL into the LC/MS system. Plasma calibrators (0.5–2000 ng/mL) and quality control samples were prepared by dilution of working standard solutions with drug-free equine serum.

Quantitative analysis of serum was performed on a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA, USA) coupled with a turbulent flow chromatography system (TFC TLX2; Thermo Scientific) having LC-10ADvp liquid chromatography systems (Shimadzu, Kyoto, Japan) and operated in laminar flow mode. Product masses and collision energies of each analyte were optimized by infusing the standards into the TSQ Vantage. Chromatography employed an ACE 3 C18 10 cm × 2.1 mm column (Mac-Mod Analytical, Chadds Ford, PA, USA) and a linear gradient of ACN in water with a constant 0.2% formic acid at a flow rate of 0.30 mL/min. The initial ACN concentration was held at 5% for 0.5 min, ramped to 45% over 5 min, increased to 95% over 1 min, held at that concentration for 0.3 min, before re-equilibrating for 4.3 min at initial conditions.

Detection and quantification was conducted using selective reaction monitoring of initial precursor ion for guaifenesin (mass to charge ratio (m/z) 199.213) and the internal standard guaifenesin-d_5 ((m/z) 204.142). The response for the product ion for guaifenesin ((m/z) 122.2) and the internal standard guaifenesin-d_5 ((m/z) 168.0) were plotted and peaks at the proper retention time integrated using Quanbrowser software (Thermo Scientific). Guaifenesin was quantitated by linear regression analysis. A weighting factor of 1/X was used for all calibration curves.

The response for guaifenesin was linear and gave correlation coefficients (R^2) of 0.99 or better. Accuracy was reported as percent nominal concentration, and precision was reported as percent relative standard deviation. The technique was optimized to provide a limit of quantitation (LOQ) of 0.5 ng/mL and a limit of detection (LOD) of approximately 0.3 ng/mL for guaifenesin.

Noncompartmental analysis was used for determination of pharmacokinetic parameters for guaifenesin using commercially available software (Phoenix WinNonlin Version 6.3; Pharsight, Cary, NC, USA). The area under the curve was calculated using the log-linear trapezoidal rule and extrapolation to infinity using the last measured plasma concentration divided by the terminal slope λ_z.

Accuracy and precision for the assay was considered acceptable based on Food and Drug Administration guidelines for bioanalytical method validation (Table 1). The semi-log plot of serum guaifenesin concentrations over time is depicted in Fig. 1. Pharmacokinetic parameters following guaifenesin administration are listed in Table 3. C_{max}, C_{min}, and C_{mean} concentrations varied widely between horses. The AUC did not increase substantially between the first and last dose as indicated by an accumulation index of 1.04 (median). The serum elimination half-life of guaifenesin ranged between 1.32 and 4.77 h. Guaifenesin serum concentrations were below the LOQ of the assay by 36 h following the final dose administration (84 h following dose 1) in six horses. Serum concentrations were above the LOQ of the assay at 48 h (96 h following dose 1) in the remaining three horses (Table 2).

Table 1. Accuracy and precision values for LC-MS/MS analysis of guaifenesin in equine serum

Concentration (ng/mL)	Intraday accuracy (% nominal conc)	Intraday precision (% relative SD)	Interday accuracy (% nominal conc)	Interday precision (% relative SD)
1.5	99.0	6.0	94.0	9.0
80.0	104	4.0	105	4.0
1600	89.0	4.0	91.0	5.0

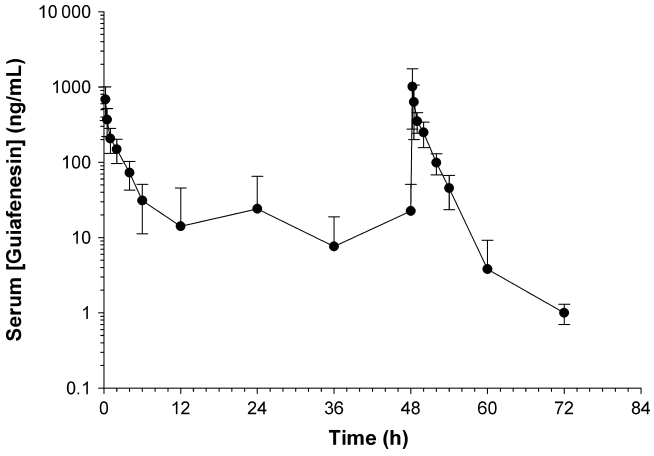

Fig. 1. Average ± SD serum guaifenesin concentrations following oral administration of 2 g BID for five doses to nine exercised Thoroughbred horses. Only values >LOQ are shown.

Table 2. Mean ± SD, median and range of serum guaifenesin concentrations at various time postoral administration of 2 g BID for five doses to nine exercised Thoroughbred horses. Times are postadministration of the first dose

Time (h)	Mean ± SD (ng/mL)	Median (ng/mL)	Range (ng/mL)
0	ND	ND	ND
0.25	681.3 ± 323.8	568.6	147.6–1178.4
0.50	367.3 ± 147.5	374.5	106.0–587.1
1.0	205.9 ± 74.6	202.0	66.1–282.5
2.0	148.8 ± 52.8	148.1	93.9–240.5
4.0	72.6 ± 30.0	68.5	36.8–100.4
6.0	31.0 ± 19.8	27.8	10.0–59.6
12	14.1 ± 31.6	1.7	<LOD–96.8
24	24.0 ± 40.9	5.9	2.6–128.3
36	7.6 ± 11.3	3.3	1.2–35.7
48	22.5 ± 28.4	10.0	3.4–81.6
48.25	1008.0 ± 732.8	776.8	405.4–2883.6
48.5	630.1 ± 430.1	503.2	217.0–1729.5
49	349.3 ± 106.3	334.5	195.9–514.0
50	248.4 ± 92.2	232.7	112.2–373.1
52	98.6 ± 30.7	100.8	49.5–157.4
54	45.2 ± 21.7	43.4	17.6–157.4
60	3.8 ± 5.4	2.1	1.2–18.2
72	1.0 ± 0.3	1.0	<LOD–1.5
84	0.3 ± 0.2	0.1	<LOD–0.9
96	0.3 ± 0.4	0.1	<LOD–0.9

Table 3. Pharmacokinetic parameters for guaifenesin following oral administration of 2 g BID for five doses to nine exercised Thoroughbred horses. All values in this table were generated using noncompartmental analysis

	Mean (±SD)	Median	Range
T_{max} (h)			
After 1st dose	0.25 ± 0.0	0.25	0.25–0.25
After last dose	0.25 ± 0.0	0.25	0.25–0.25
C_{max} (ng/mL)			
After 1st dose	681.3 ± 323.8	568.6	147.6–1089.5
After last dose	1008.0 ± 732.8	776.8	405.4–2883.6
C_{min} (ng/mL)			
After 1st dose	14.1 ± 31.6	1.7	0.3–96.8
Lambda$_z$ (1/h)	0.263 ± 0.119	0.277	0.148–0.523
Serum $t_{1/2\lambda}$ (h)‡	2.62 ± 1.24	2.49	1.32–4.77
AUC_{inf} (h·ng/mL)	1445.9 ± 398.3	1554.1	971.2–2270.2
Accumulation index	1.09 ± 0.08	1.04	1.00–1.21

T_{max}, time to maximal plasma concentration; C_{max}, maximal plasma concentration; C_{min}, minimal plasma concentration; $t_{1/2\lambda}$, half-life λ_z; AUC_{inf}, area under the plasma concentration–time curve from time 0 to infinity. Accumulation index was determined by use of the equation (AUC[12], τ)/(AUC[1, τ]), where dose 5 and 1 are the last and first dose, respectively. ‡Harmonic mean.

While scientific studies describing the effectiveness of guaifenesin as an expectorant in veterinary species are lacking, anecdotal reports of its usefulness for this indication make its use commonplace in performance horses. While only injectable formulations are approved for use in veterinary species, oral guaifenesin products, including powders and pastes, are widely available from compounding pharmacies. As the use of this drug is regulated in racehorses and to the best of the authors' knowledge, there are no reports describing the disposition of guaifenesin following multiple oral administrations, the primary objective of the current study was to describe the pharmacokinetics of guaifenesin in horses in order to provide data upon which appropriate regulatory recommendations can be made.

Guaifenesin was rapidly absorbed following oral administration, with peak concentrations achieved within 15 min of the initial administration. Similarly, maximum concentrations were achieved within 15 min following the final dose. The fluctuation in guaifenesin concentrations (difference between C_{max} and C_{min}) within dosing intervals was large, with very low levels of drug measured immediately prior to subsequent dose administration. As expected for a drug with a dosing interval much longer than its elimination half-life, bioaccumulation of guaifenesin was not observed in the current study.

The terminal plasma half-life of guaifenesin in the current study was 2.62 ± 1.24 h, which is slightly prolonged compared to a previous report describing intravenous administration to horses (1.77 ± 0.32 h; Matthews *et al.*, 1997). This difference is likely attributable to the more sensitive analytical assay utilized in the current study (LOQ of 0.1 ng/mL) as compared to the previous study (LOQ: 700 ng/mL; Matthews *et al.*, 1997) or differences in sampling protocols. Blood samples were collected for a prolonged period of time in the current study (48 h postadministration of the final dose) relative to the study by Matthews *et al.* (1997) (6 h following termination of the infusion). Being able to quantitate concentrations for a longer period of time requires less extrapolation of the terminal elimination portion of the plasma concentration–time curve, likely allowing for a more accurate calculation of the elimination half-life.

Guaifenesin is commonly used in racehorses to aid in the clearance of mucus from the airways and as such drug

withdrawal is necessary prior to competition. The presently reported study provides data that can be utilized to establish appropriate regulatory recommendations.

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