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Elimination of cetirizine following administration of multiple doses to exercised thoroughbred horses

H. K. KNYCH*,†
S. D. STANLEY*,†
R. M. ARTHUR‡ &
D. S. MCKEMIE*

*K.L. Maddy Analytical Chemistry Laboratory, School of Veterinary Medicine, University of California, Davis, CA, USA; †Department of Veterinary Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA, USA; ‡School of Veterinary Medicine, University of California, Davis, CA, USA

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Cetirizine is an antihistamine used in performance horses for the treatment of hypersensitivity reactions and as such a withdrawal time is necessary prior to competition. The objective of the current study was to describe the disposition and elimination of cetirizine following oral administration in order to provide additional serum concentration data upon which appropriate regulatory recommendations can be established. Nine exercised thoroughbred horses were administered 0.4 mg/kg of cetirizine orally BID for a total of five doses. Blood samples were collected immediately prior to drug administration and at various times postadministration. Serum cetirizine concentrations were determined and selected pharmacokinetic parameters determined. The serum elimination half-life was 5.83 ± 0.841 h. Average serum cetirizine concentrations were still above the LOQ of the assay (0.05 ng/mL) at 48 h (final sample collected) postadministration of the final dose.

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Heather Knych, K.L. Maddy Analytical Chemistry Laboratory, School of Veterinary Medicine, University of California, Davis, CA 95616, USA.
E-mail: hkknych@ucdavis.edu

Cetirizine is a nonsedative second-generation H1 receptor antagonist that is used sporadically in veterinary medicine for the treatment of hypersensitivity reactions. Arguably hydroxyzine, a pharmacologically active prodrug of cetirizine, is more commonly prescribed in equine medicine but due to its sedative properties and classification by the Association of Racing Commissioners International (ARCI) as a class 2 drug under the Uniform Classification Guidelines for Foreign Substances, cetirizine, a class 4 foreign substance, may be a more prudent choice for racehorses. While the pharmacokinetics and pharmacodynamics of cetirizine in horses has been reported previously (Olsen et al., 2007, 2008, 2011), serum concentration data upon which to make regulatory recommendations are limited. The goal of the currently reported study was to expand upon and add to existing reports describing cetirizine serum concentrations 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.

Nine healthy exercised adult thoroughbred horses including 4 geldings and 5 mares (4–9 years of age; weight of 474–590 kg) were studied. Horses are exercised on a continual basis and exercise continued throughout the study. The exercise regimen consists of 6 days a week of exercise 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).

Prior to drug administration, a 14-gauge catheter was aseptically placed in one external jugular vein for sample collection. Cetirizine tablets (Cetirizine HCL; Perrigo, Allegan, MI, USA) were administered suspended in water (60 mL) into the oral cavity via a dosing syringe at a dose of 0.4 mg/kg BID for a total of five doses. Dose selection was determined based on an informal survey of equine practitioners conducted by the Racing Medication and Testing Consortium.

Blood samples were collected at time 0, 1, 2, 4, 6, and 12 h after the first dose and at 1, 2, 4, 6, 12, 24, 36, and 48 h following the final dose. Additional samples were collected at 12-hour interval 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 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, 0.5 mL of serum was diluted with 100 μL of water containing 25 ng/mL of d8-cetirizine internal standard (Toronto Research Chemicals; Toronto, ON, Canada) and 2 mL of 0.6 M pH 6.5 phosphate buffer. The samples were vortexed briefly to mix and subjected to solid phase extraction using Cerex polychrom Clin II 3 cc 35 mg columns (Cera, Inc. Baldwin Park, CA, USA). In brief, samples were loaded onto the columns and then washed consecutively with 3 mL each of water, 2 mL of 1.0 M acetic acid, and 3 mL of methanol prior to elution with 3 mL of methylene chloride:2-propanol: ammonium hydroxide (78:20:2 v:v:v). Samples were dried under nitrogen and dissolved in 150 μL of 5% acetonitrile (ACN) in water, both with 0.2% formic acid. The injection volume was 30 μL. Plasma calibrators (0.001–100 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 TLX4; Thermo Scientific) having 1100 series liquid chromatography systems (Agilent Technologies, Palo Alto, CA, USA) and operated in laminar flow mode. Product masses and collision energies were optimized by directly infusing the standards into the mass spectrometer. Chromatography employed an ACE 3 C18 100 × 2.1 mm, 3 μm column (Mac-Mod Analytical, Chadds Ford, PA, USA) and a linear gradient of acetonitrile (ACN) in water, both with 0.2% formic acid, at a flow rate of 0.40 mL/min. The initial ACN concentration was held at 5% for 0.5 min, ramped to 99% over 6 min, held at that concentration for 1 minute, before re-equilibrating for 4.3 min at initial conditions.

Detection and quantification was conducted using selective reaction monitoring of initial precursor ion for cetirizine (mass-to-charge ratio *(m/z)* 389.154) and the internal standard d8-cetirizine (*(m/z)* 397.215). The response for the product ions for cetirizine (*m/z* 166.1) and the internal standard d8-cetirizine (*m/z* 165.1, 201.1) was plotted, and peaks at the proper retention time were integrated using Quanbrowser software (Thermo Scientific). Cetirizine was quantitated by linear regression analysis. A weighting factor of 1/X was used for all calibration curves.

The response for cetirizine 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 of 0.05 ng/mL and a limit of detection of approximately 0.02 ng/mL for cetirizine.

Noncompartmental analysis was used for determination of pharmacokinetic parameters for all compounds using commercially available software (Phoenix WinNonlin Version 6.3; Pharsight, Cary, NC, USA). $Lambda_z$ was calculated using time points ranging from 51.3 ± 1.4 to 96.0 ± 0 h. The area under the curve within a dosing interval (tau) was calculated using the log-linear trapezoidal rule. The accumulation index was determined using the equation (AUC[12], τ)/(AUC[1, τ]), where doses 5 and 1 are the last and first doses, respectively.

Accuracy and precision for both assays were considered acceptable based on Food and Drug Administration guidelines for Bioanalytical Method Validation (Table 1). The semi-log plot of serum cetirizine concentrations over time is depicted in Fig. 1. Mean (±SD) serum cetirizine concentrations are listed in Table 2 and selected pharmacokinetic parameters in Table 3. Accumulation of cetirizine following multiple administrations was minimal as indicated by the calculated accumulation ratio of 1.30 (median). The serum elimination half-life ranged from 4.88 to 7.81 h. Serum cetirizine concentrations were still above the LOQ (0.05 ng/mL) of the assay at 48 h post-administration of the last dose in all horses studied.

The effectiveness of the antihistamine, cetirizine, in treating hypersensitivity reactions in horses has been demonstrated (Olsen et al., 2008, 2011). As cetirizine is used in performance horses, the primary goal of this study was to generate additional cetirizine serum concentration data to establish regulatory recommendations for race and performance horses. As regulatory recommendations rely heavily on terminal drug

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

Concentration (ng/mL)	Intraday accuracy (% nominal conc)	Intraday precision (% relative SD)	Interday accuracy(% nominal conc)	Interday precision (% relative SD)
0.15	114	4.0	114	6.0
75.0	100	4.0	98.0	3.0
200	94.0	5.0	95.0	4.0

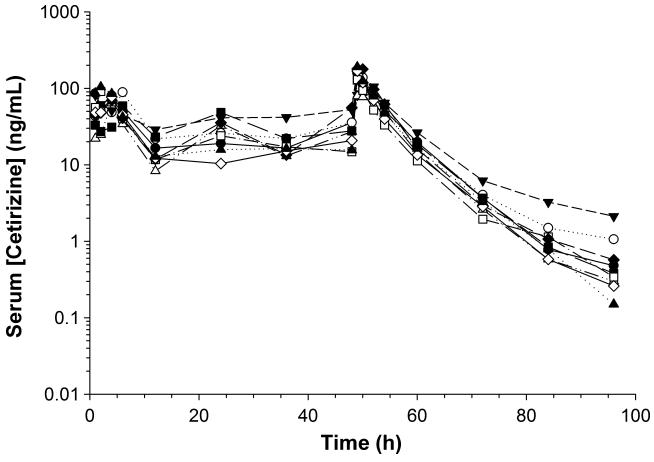

Fig. 1. Serum cetirizine concentrations following oral administration of 0.4 mg/kg cetirizine BID for a total of five doses to nine exercised thoroughbred horses.

Table 2. Mean ± SD, median and range of serum cetirizine concentrations at various times post oral dose of 0.4 mg/kg BID for five doses to nine exercised thoroughbred horses

Time (h)	Mean ± SD (ng/mL)	Median (ng/mL)	Range (ng/mL)
0	ND	ND	ND
1.0	55.6 ± 23.7	48.3	22.0–86.4
2.0	57.0 ± 26.3	50.3	24.7–104.2
4.0	64.1 ± 17.5	69.5	31.0–85.1
6.0	49.4 ± 16.3	45.0	34.2–88.7
12	16.3 ± 6.75	12.9	8.29–28.8
24	28.0 ± 12.4	25.9	10.3–48.2
36	19.7 ± 8.85	16.6	13.1–22.0
*48	31.7 ± 14.8	27.7	14.8–52.8
49	142.2 ± 40.6	158.2	79.1–190.1
50	116.6 ± 29.2	118.4	76.9–177.3
52	80.5 ± 15.3	78.7	51.9–104.5
54	50.9 ± 10.3	51.0	33.0–64.2
60	17.1 ± 4.37	16.3	11.2–26.4
72	3.46 ± 1.21	3.28	1.94–6.20
84	1.17 ± 0.833	0.844	0.58–3.25
96	0.628 ± 0.618	0.379	0.15–2.12

*Last dose given.

Table 3. Pharmacokinetic parameters for cetirizine following oral dose of 0.4 mg/kg BID for five doses to nine exercised thoroughbred horses. All values in this table were generated using noncompartmental analysis

Parameter	Mean (±SD)	Median	Range
$Lambda_z$ (1/h)	0.118 ± 0.015	0.123	0.089–0.142
Serum $t_{1/2\lambda}$ (h)*	5.83 ± 0.841	5.66	4.88–7.81
AUC_{tau} (h ng mL)	724 ± 114	753	529–922
C_{avg} (ng/mL)	60.4 ± 9.52	62.8	44.1–76.9
C_{last} (ng/mL)	0.629 ± 0.618	0.38	0.15–1.06
Accumulation Index	1.33 ± 0.088	1.30	1.28–1.53

$Lambda_z$, slope of the terminal portion of the serum concentration curve; $t_{1/2\lambda}$, half-life λz; AUC_{tau}, area under the plasma concentration –time curve over the nth dosing interval from time 0 to the time τ after dose; C_{avg}, average steady-state serum concentration; C_{last}, last measured serum concentration.

Accumulation index was determined using the equation (AUC[12], τ)/(AUC[1, τ]), where doses five and one are the last and first doses, respectively.

*Harmonic mean.

concentrations, for this study, we chose to focus primarily on cetirizine elimination pharmacokinetics.

The median terminal half-life of cetirizine in the current study was 5.66 h, which is in close agreement with a previous report utilizing the same dose (5.8 h; Olsen et al., 2008) but slightly prolonged compared with another study by the same group of investigators in which a sparse sampling method was used (3.5 h; Olsen et al., 2011). The discrepancy in the terminal half-life between studies is likely attributable to differences in the samples included in the pharmacokinetic analysis. In the second study conducted by Olsen *et al.* (2011), only samples collected up to 24 h postadministration of the final dose were included in the analysis. In the current study as well as the first study by Olsen *et al.* (2008), samples collected up to 48 h and 30 h, respectively, were included in the pharmacokinetic analysis.

Olsen *et al.* (2007) also assessed the effects of ivermectin pretreatment on the elimination of cetirizine in horses. When cetirizine was administered 12 h postivermectin treatment, the elimination half-life was significantly prolonged relative to animals that only received cetirizine (Olsen *et al.*, 2007). The authors concluded that the prolonged elimination half-life in the horses receiving both compounds was attributable to P-glycoprotein inhibition by ivermectin (Olsen *et al.*, 2007). Therefore, a prolonged withdrawal time recommendation may be prudent when cetirizine is used in combination with ivermectin or any other P-glycoprotein inhibitor.

Cetirizine is prescribed by some veterinarians for use race and performance horses to treat hypersensitivity reactions 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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