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RESEARCH ARTICLE

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Detection and pharmacokinetics of grapiprant following oral administration to exercised Thoroughbred horses

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Abstract

Traditional therapeutic options for the treatment of lameness associated with inflammation in performance horses include administration of cyclooxygenase enzyme inhibiting non-steroidal anti-inflammatory drugs (NSAID). As long-term use of these drugs can adversely impact the health of the horse, anti-inflammatories with a more favorable safety profile are warranted. Grapiprant is a newly approved non-cyclooxygenase inhibiting NSAID that has demonstrated efficacy and safety in other species and which may be a valuable alternative to traditional NSAIDs used in the horse. The objectives of the current study were to describe drug concentrations and the pharmacokinetics of grapiprant in exercised Thoroughbred horses and to develop an analytical method that could be used to regulate its use in performance horses. To that end, grapiprant, at a dose of 2 mg/kg was administered orally to 12 exercised Thoroughbred horses. Blood and urine samples were collected prior to and for up to 96 hours post drug administration. Drug concentrations were measured using liquid chromatography–tandem mass spectrometry. Grapiprant remained above the LOQ of the assay (0.005 ng/mL) in serum for 72 hours post administration and urine concentrations were above the LOQ until 96 hours. The C_{max}, T_{max} and elimination half-life were 31.9 ± 13.9 ng/mL, 1.5 ± 0.5 hours and 5.86 ± 2.46 hours, respectively. The drug was well tolerated in all horses at a dose of 2 mg/kg. Results support further study of this compound in horses. Furthermore, development of a highly sensitive analytical method demonstrate that this compound can be adequately regulated in performance horses.

KEYWORDS

grapiprant, horse, horse racing, NSAID, pharmacokinetics

1 | INTRODUCTION

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are arguably the most commonly used class of drugs in equine medicine and remain the mainstay of treatment for horses with musculoskeletal pain and inflammation.[1] This class of drugs act by inhibiting cyclooxygenase enzymes (COX-1 and COX-2), thereby decreasing the production of a number of eicosanoids, including prostaglandins, leukotrienes, and thromboxane A_2, all of which play a key role in the inflammatory cascade. These inflammatory mediators are responsible for the sequelae of inflammation including increased vascular permeability, heat, and decreased nociceptor thresholds. Along with inhibition of the production of inflammatory mediators through a blockade of COX enzymes, comes inhibition of mediators responsible for functions associated with cellular homeostasis. Inhibition of the latter is at least partially responsible for the adverse effects associated with NSAID administration. This includes sequelae such as gastric ulceration, right dorsal colitis, renal toxicity, and suppressed bone and wound healing.

Although newer COX-2 selective NSAIDs, such as firocoxib, appear to have fewer adverse effects than more traditional non-selective NSAIDs, they are not completely devoid of untoward effects.

Recently, a new non-COX enzyme inhibiting NSAID was approved for use in veterinary medicine. By virtue of its mechanism of action, this drug would appear to have fewer adverse effects than the COX enzyme inhibiting drugs such as phenylbutzone and flunixin meglumine. Grapiprant (Galliprant®) (Figure 1) is approved for use in dogs and is classified as a prostaglandin E2 (PGE2) receptor antagonist.[2] To date, 4 PGE2 receptor subtypes (EP1–EP4) have been identified.[3] Grapiprant binds to the EP4 receptor, blocking the PGE2 mediated sensitization of sensory neurons and stimulation of inflammation, controlled through this receptor.[3-7] As it does not inhibit COX enzymes, presumably homeostatic functions associated with COX enzymes would be preserved.[2] Rausch-Derra et al. reported that grapiprant is an effective treatment for alleviation of pain in dogs with osteoarthritis and appears to be better tolerated than other treatments following administration of 2 mg/kg daily for 28 days.[2] Although not approved for use in cats, investigators have also reported that grapiprant is well tolerated when administered at doses of 3, 9, and 15 mg/kg daily for 28 days to this species.[8] To the best of our knowledge, there are no reports in the literature describing the use of grapiprant in horses; however, if this drug proves to be effective and safe, it may provide veterinarians with an additional therapeutic option for the treatment of inflammation in horses.

As there are no reports of the use of grapiprant in horses, in this initial study we conducted a study to describe drug concentrations and pharmacokinetic using the manufacturer's recommended dose for dogs. As this drug is an anti-inflammatory, it also has the potential to be used in performance horses and therefore a secondary goal of this study was to develop an analytical method that could be used to regulate its use in horses, similar to what has been reported for other species.[9]

2 | MATERIALS AND METHODS

2.1 | Horses

Twelve healthy, University-owned and exercised Thoroughbred research horses (8 mares and 4 geldings, age 2–6 years; weight (mean ± SD) 499.9 ± 46.6 kg) were utilized for the current study. All horses were exercised 5 days a week prior to and throughout the study, with the exception of the day of drug administration, according to standard laboratory protocols.[10] Three days prior to commencement of the study, all horses were determined healthy and free of disease by physical examination, complete blood count, and a serum biochemistry panel that included aspartate aminotransferase, creatinine phosphokinase, alkaline phosphatase, total bilirubin, sorbital dehydrogenase, blood urea nitrogen, and creatinine. All blood analyses were performed by the Clinical Pathology Laboratory of the William R. Pritchard Veterinary Medical Teaching Hospital of the University of California, Davis, using their standard protocols. Water was available ad libitum throughout the study. No medications were administered to any horses for at least 4 weeks prior to the study. This study was approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

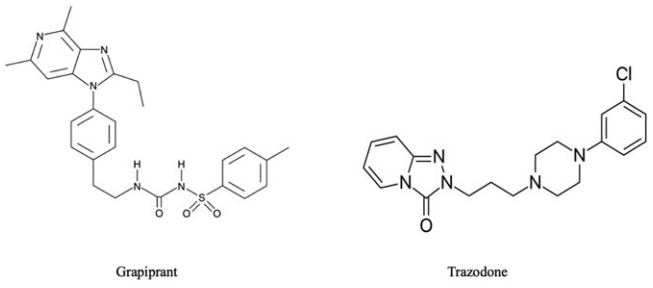

FIGURE 1 Chemical structures of grapiprant and trazodone

2.2 | Instrumentation and drug administration

Horses were fasted for 12 hours prior to and 4 hours following drug administration. Thereafter, food was available ad libitum for the remainder of the study. Each horse was weighed the morning of drug administration and a 14-guage catheter placed, using aseptic technique, in 1 external jugular vein for sample collection. All horses received a single oral administration of 2 mg/kg grapiprant tablets (Galliprant®; Aratana Therapeutics, Leawood, KS, USA), suspended in water. The suspension was delivered over the tongue via a dosing syringe.

2.3 | Sample collection and behavioral observations

Blood samples for grapiprant concentration determination were collected at time 0 (immediately prior to drug administration) and at 15, 30, and 45 minutes and 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 18, 24, 36, 48, 72, and 96 hours post drug administration. Prior to drawing each sample, 10 mL of blood was aspirated from the catheter and T-port extension set (combined internal volume < 2 mL) and discarded. After sample collection, the catheter was flushed with 10 mL of a dilute heparinized saline solution (10 IU/mL). Sample collection catheters were removed following collection of the 24-hour sample and the remaining samples collected by direct venipuncture. Blood samples were collected into serum tubes and placed at room temperature prior to centrifugation at 3000 x g for 10 minutes. Following centrifugation, serum was immediately transferred into storage cryovials and stored at −20°C until analyzed (approximately 2 weeks).

Urine samples were collected at time 0 (immediately prior to drug administration) and 24, 48, 72, and 96 hours post drug administration. All urine samples were collected by free catch.

Horses were observed by the investigators following drug administration and throughout the course of the sample collection for signs of distress or discomfort. Any adverse effects were noted.

2.4 | Determination of grapiprant serum and urine concentrations

The analytical reference standard for grapiprant (Figure 1) was obtained from Cayman Chemical Company (Ann Arbor, MI, USA) and the internal standard trazodone (Figure 1) was obtained from Sigma Aldrich (St Louis, MO, USA). Stock solutions of grapiprant and internal

standard were prepared at 1 mg/mL in acetonitrile (ACN) and methanol, respectively; free base. Acetonitrile and water were purchased from Burdick and Jackson (Muskegon, MI, USA). Methanol, methylene chloride, 2-propanol, ammonium hydroxide, glacial acetic acid, and buffer reagents were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Formic Acid, 98%, was purchased from Sigma Aldrich (St Louis, MO, USA). The solvents were HPLC grade or better.

2.4.1 | Serum sample analysis

Grapiprant working solutions were prepared by dilution of the 1 mg/mL stock solution with methanol to concentrations of 0.001, 0.01, 0.1, 1, and 10 ng/μL. Serum calibrators were prepared by dilution of the working standard solutions with drug free equine serum to concentrations ranging from 0.005 to 100 ng/mL. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality control samples (blank equine serum fortified with analyte at 3 concentrations within the standard curve) were included with each sample set as an additional check of accuracy.

Prior to analysis, 500 μL of serum was diluted with 500 μL of ACN:1 M acetic acid (9:1, v:v) containing 50 ng/mL of trazodone internal standard, to precipitate proteins. The samples were vortexed for 1 minute to mix, refrigerated for 20 minutes, vortexed for an additional 1 minute, centrifuged in a Sorvall ST 40R centrifuge (Thermo Scientific, San Jose, CA, USA) at 4300 rpm (4031 x g) for 10 minutes at 4°C and 30 μL injected into the liquid chromatography– tandem mass spectrometry (LC–MS/MS) system. The concentration of grapiprant was measured in serum by LC–MS/MS using positive heated electrospray ionization. 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 (TLX2 Thermo Scientific, San Jose, CA, USA) having LC-10ADvp liquid chromatography systems (Shimadzu, Kyoto, Japan) and operated in laminar flow mode. The spray voltage was 3500 V, vaporizer temperature was 376°C, and the sheath and auxiliary gas were 45 and 25, respectively (arbitrary units). Product masses and collision energies of each analyte were optimized by infusing the analytes into the mass spectrometer. 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, both with 0.2% formic acid, at a flow rate of 0.35 ml/min. The initial ACN concentration was held at 5% for 0.33 minutes, ramped to 95% over 5 minutes and held at that concentration for 0.17 minutes, before re-equilibrating for 3.5 minutes at initial conditions.

Detection and quantification was conducted using selective reaction monitoring (SRM) of initial precursor ion for grapiprant (mass to charge ratio (*m/z*) 492.2) and trazodone (*m/z* 372.2). The product ions used for grapiprant quantitation (*m/z* 174.2, 321.2) were chosen to optimize signal to noise levels. The grapiprant and internal standard product quantifier ions (*m/z* 78.3, 148.1, 176.0) were plotted and peaks at the proper retention time integrated using Quanbrowser software (Thermo Scientific, San Jose, CA, USA). Quanbrowser software was used to generate calibration curves and quantitate grapiprant in all samples by linear regression. A weighting factor of 1/X was used for all calibration curves.

2.4.2 | Urine sample analysis

Working solutions were the same as for the serum analysis. Urine calibrators were prepared by dilution of the working standard solutions with drug-free equine urine to concentrations ranging from 0.1 to 500 ng/mL. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality control samples (drug-free equine urine fortified with analyte at 3 concentrations within the standard curve) were included with each sample set as an additional check of accuracy.

One milliliter of urine was diluted with 0.2 mL of water containing 40 ng/mL of trazadone internal standard and 0.4 mL of β-glucuronidase enzyme, (*Patella vulgata*, Sigma Aldrich, St Louis, MO, USA) at 10,000 Units/mL in pH 5, 1.6 M acetate buffer. The pH of the samples was adjusted to 5 ± 0.5 with 2 N NaOH or 2 N HCl, as necessary, and heated in a sonicating water bath at 65°C for 2 hours with 99 minutes of sonication. After cooling to room temperature, the pH was adjusted to 6.0 ± 0.5 with 1.5 mL of pH 6.5, 0.6 M phosphate buffer and 2 N NaOH or 2 N HCl, as necessary. Samples were mixed gently and centrifuged at 4000 rpm (3488 x g) for 5 minutes at 4°C. The samples were subjected to solid-phase extraction (SPE) using CleanScreen extraction columns DAU (130 mg, 3 mL) (United Chemical Technologies, Bristol, PA, USA). In brief, the columns were conditioned with 3 mL of methanol and 3 mL of 0.1 M pH 6 phosphate buffer before the samples were loaded onto the column. The samples were passed through the columns using a CEREX system 48 Processor with positive pressure SPE manifold (SPE Ware, Baldwin Park, CA, USA); no less than 2 minutes was allowed for samples to pass through the column. The columns were rinsed consecutively with 3 mL of water, 2 mL of 1 M acetic acid, 3 mL of methanol, prior to elution with 2.5 mL (78:20:2, v:v:v) methylene chloride:2-propanol:ammonium hydroxide. The samples were then dried under nitrogen in a Zymark TurboVap evaporator (McKinley Scientific, Sparta, NJ, USA) at 45°C and reconstituted in 200 μL of 5% acetonitrile in water, both with 0.2% formic acid and 20 μL was injected into the LC–MS/MS system. Detection and quantification was the same as described for serum.

2.5 | Pharmacokinetic calculations

Non-compartmental analysis was used for determination of pharmacokinetic parameters for grapiprant using commercially available software (Phoenix WinNonlin Version 6.3, Pharsight, Cary, NC). The terminal-phase half-life (λ *z* HL), the area under the curve from time 0 to infinity ($AUC_{0 \rightarrow \infty}$) and the extrapolated percentage of the area under the curve (AUC %) were determined. The λ *z* HL was calculated using the $t_{1/2} = 0.693/ \lambda z$ equation and area under the curve and area under the moment curve were calculated using the log up-linear down trapezoidal method and extrapolated to infinity using the last measured serum concentration divided by the terminal slope λ_z.

3 | RESULTS

The response for grapiprant in serum and urine was linear and gave correlation coefficients (R^2) of 0.99 or better. The intra-day, inter-day, analyst-to-analyst precision and accuracy of the assay were

determined by assaying quality control samples in replicates (n = 6) for grapiprant. Accuracy was reported as percent nominal concentration and precision was reported as percent relative standard deviation (Table 1). Accuracy and precision for all matrices were considered acceptable based on the Food and Drug Administration's guidelines for Bioanalytical Method Development.[11] The presence of grapiprant in the post-administration serum and urine samples, was determined by matching the retention times as well as relative abundances of 2 quantifier ions (m/z 174.2, 321.2) in the SRM ion chromatogram MS/MS spectra (Figure 2). The limit of quantitation (LOQ) was the lowest calibrator that could be measured with acceptable precision and accuracy.[11] The limit of detection (LOD) was established based on the lowest calibrator with a 3:1 signal-to-noise ratio. The technique was optimized to provide an LOQ of 0.005 ng/mL and an LOD of approximately 0.0025 ng/mL in serum and an LOQ of 0.1 ng/mL and LOD of approximately 0.005 ng/mL in urine. The SPE recovery for both grapiprant and trazodone was approximately 90%.

Grapiprant administration was well tolerated in all horses and no untoward effects were noted. Individual serum grapiprant concentration over time curves are depicted in Figure 3 and the mean (±SD) serum grapiprant concentrations are listed in Table 2. Grapiprant concentrations were below the LOQ (0.005 ng/mL) in serum by 72 hours in 6/12 horses and 3/12 at 96 hours. Serum grapiprant concentrations were below the LOQ but above the LOD (0.0025 ng/mL) at 72 hours in 6/12 horses and still above the LOD in 3 of these horses at 96 hours. Urine grapiprant concentrations ranged from 0.1 to 7.29 ng/mL at the last time point collected (96 hours) (Table 2). Individual horse and summary pharmacokinetic parameters for serum are listed in Table 3.

TABLE 1 Accuracy and precision values for LC–MS/MS analysis of grapiprant in equine serum and urine. Values represent the average of 6 replicates at each concentration for each biological matrix

Matrix	Concentration (ng/mL)	Intra-day Accuracy (% Nominal Concentration)	Intra-day Precision (% relative SD)	Inter-day Accuracy (% Nominal Concentration)	Inter-day Precision (% Relative SD)
Serum					
	0.015	94.0	14.0	99.0	14.0
	10.0	112	4.0	110	5.0
	50.0	106	8.0	104	8.0
Urine					
	0.30	104	3.0	104	4.0
	10.0	103	2.0	99.0	3.0
	200	105	3.0	106	4.0

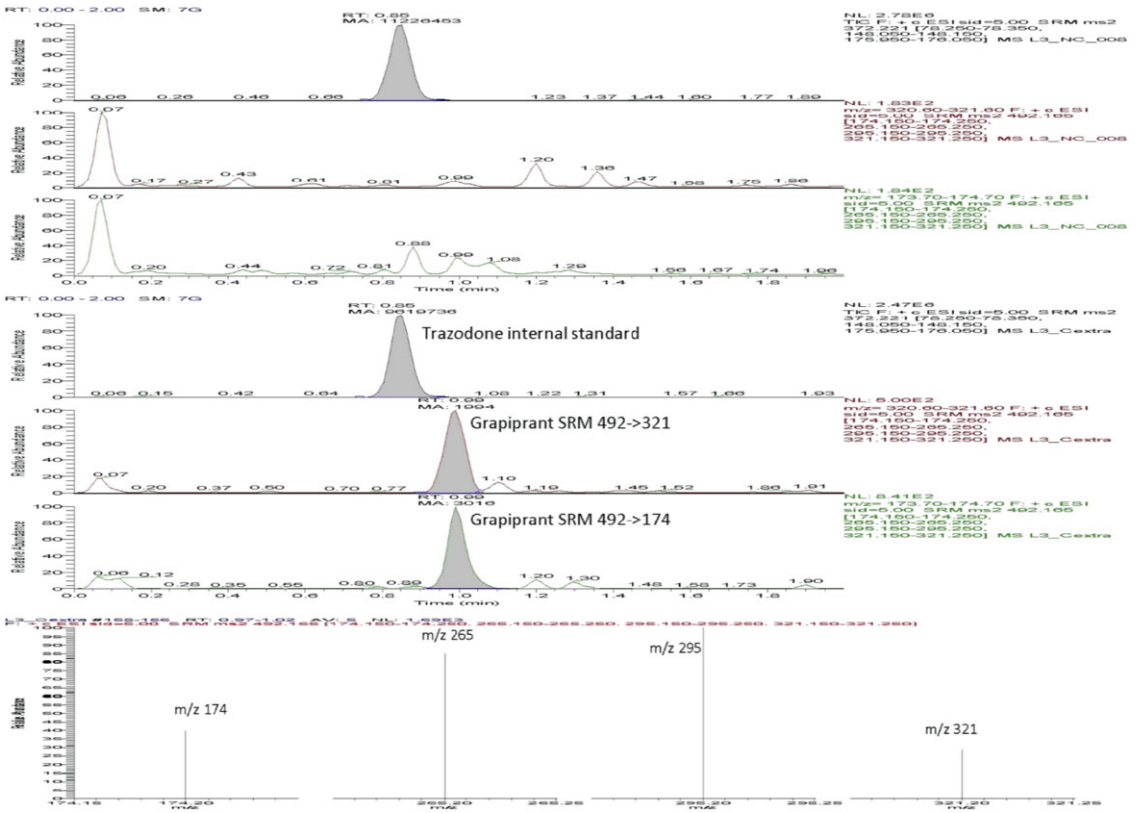

FIGURE 2 Product ion MS chromatogram and spectra of grapiprant and internal standard spiked at the method LOQ in equine serum [Colour figure can be viewed at wileyonlinelibrary.com]

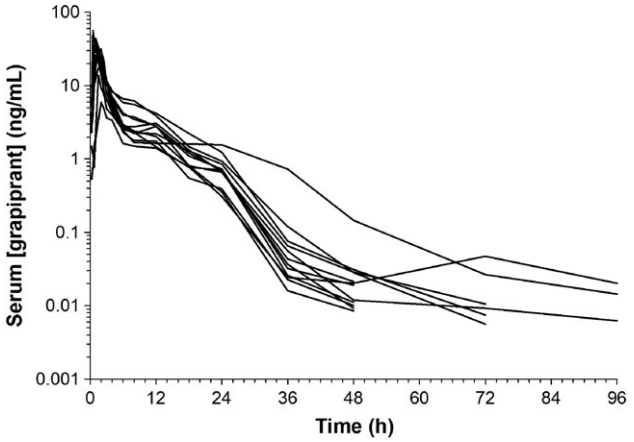

FIGURE 3 Individual grapiprant serum concentration curves following administration of 2 mg/kg grapiprant to 12 exercised Thoroughbred horses

TABLE 2 Mean (± SD) serum and urine grapiprant concentrations following a single oral administration of 2 mg/kg of grapiprant tablets (Galliprant®) to 12 exercised Thoroughbred horses

Time (hr)	[Serum] (ng/mL)	[Urine] (ng/mL)
0	ND	ND
0.25	3.87 ± 2.55	---
0.5	10.6 ± 15.0	---
0.75	19.5 ± 13.6	---
1	24.3 ± 14.1	---
1.5	24.6 ± 9.02	---
2	20.1 ± 8.26	---
2.5	13.7 ± 5.65	---
3	9.03 ± 2.77	---
4	5.81 ± 1.63	---
6	3.42 ± 1.50	---
8	2.99 ± 1.51	---
12	2.51 ± 0.92	---
18	1.12 ± 0.47	---
24	0.76 ± 0.36	443.3 ± 123.6
36	0.10 ± 0.20	---
48	0.03 ± 0.04 (12/12)	8.98 ± 10.5 (12/12)
72	0.02 ± 0.02 (6/12)	2.96 ± 4.66 (12/12)
96	0.01 ± 0.01 (3/12)	1.52 ± 2.37 (12/12)

ND, not detected; Number in () represents number of horses above the limit of quantitation.

4 | DISCUSSION

Grapiprant is an NSAID recently approved for use in dogs that purportedly has fewer adverse effects than traditional NSAIDs.[12] Although not currently approved for use in horses, the primary objective of the presently reported study was to describe drug concentrations in serum and urine and the pharmacokinetics of this compound in horses following oral administration. As this anti-inflammatory drug has the potential to be used in performance horses, a secondary goal was to develop an analytical method, as has been described for other species,[9] that could be used to regulate its use.

While the pharmacokinetics of grapiprant have been described in other species,[2,8,13-15] to the best of the authors' knowledge there no reports in horses. In the current study, the C_{max} ranged from 5.93 to 56.3 ng/mL following oral administration of a 2 mg/kg dose. This is much lower than what has been reported for other species following administration of an equivalent dose. In cats, C_{max} is reportedly 490–750 ng/mL.[8] Maximal plasma concentrations were 1598 ng/mL in fasted and 614 ng/mL in fed dogs following administration of an oral dose of 2 mg/kg.[15] The discrepancy between the 2 studies suggests large interspecies differences in absorption. However, it should also be noted that in the current study, drug was crushed, suspended and administered via a dosing syringe to mimic a common means of oral administration of drugs to horses. While every attempt was made to ensure that the entire dose was administered, administration via a dosing syringe does present the opportunity for loss of drug from the oral cavity. This could preclude some of the dose from reaching the gastrointestinal tract. In the study conducted by Rausch-Derra et al.,[14] the tablet was placed intact in the back of the oral cavity of dogs, ensuring that the entire dose reached the gastrointestinal tract. Unfortunately, this is not logistically possible in horses. Although not feasible under most clinical situations, future studies using a nasogastric tube to deliver the drug directly to the gastrointestinal tract may yield a better approximation of true absorption from the gastrointestinal tract of horses. Additionally, a study whereby grapiprant is administered via the intravenous route, would allow for calculation of the absolute bioavailability of this drug in horses. Food has a demonstrated effect on absorption of grapiprant in dogs, with bioavailability following administration with food estimated at 59.1% compared to 111.9% in the fasted state.[15] Based on this, in the current study, food was withheld for 4 hours following grapiprant administration. The time to maximum concentration was similar between dogs (1–2 hours),[1] cats (1.33 hours),[8] and horses (0.5–2 hours).

The elimination half-life reported for dogs range from 2.99 to 9.12 hours following a single oral administration[14] while in cats, it is 1.42–6.05 hours.[8] In the current study, the elimination half-life for horses was slightly longer, ranging from 4.06 to 11.1 hours. This could be due to differences in elimination between species but could also be attributable to differences in the duration of sample collection between the studies. In the current study, samples were collected until grapiprant concentrations were below the LOQ of the analytical assay (0.005 ng/mL). In the study conducted by Lebkowska-Wieruszewska et al., the average concentration in the final sample collected was approximately 30 ng/mL (24 hours).[8] The final measured concentration in the study conducted by Rausch-Derra et al. was approximately 10 ng/mL (36 hours).[14] It is possible, that the differences in the elimination half-life is due to differences in the amount of extrapolation of the terminal portion of the plasma concentration curve. The difference in the terminal elimination half-life reported in cats and dogs, relative to horses may be a result of a greater extrapolation in the previous studies relative to the presently reported one.

Current regulations in horse racing in the United States, allow for administration of 1 NSAID (phenylbutazone, ketoprofen or flunixin meglumine) up to 24 hours prior to post time. Although currently not permitted in horse racing, in the current study we

TABLE 3 Pharmacokinetic parameters for serum following a single oral administration of 2 mg/kg of grapiprant tablets (Galliprant®) to 12 exercised Thoroughbred horses. All values in this table were generated using non-compartmental analysis

Parameter	C_{max} (ng/mL)	T_{max} (h)	$Lambda_z$ (1/h)	Half-life $Lambda_z$ (h)	AUC_{inf} (ng*h/mL)	AUC Extrap (%)
Horse 1	13.8	1.5	0.064	10.8	78.8	0.28
Horse 2	43.8	1.0	0139	4.98	117.3	0.12
Horse 3	56.3	0.5	0.171	4.06	123.6	0.04
Horse 4	29.0	1.5	0.079	8.82	96.0	0.08
Horse 5	32.2	1.5	0.168	4.14	120.4	0.05
Horse 6	41.3	1.0	0.096	7.21	98.6	0.11
Horse 7	31.6	2.0	0.143	4.85	95.3	0.07
Horse 8	30.4	2.0	0.146	4.76	97.2	0.08
Horse 9	17.6	2.0	0.129	5.35	79.0	0.21
Horse 10	41.2	1.0	0.115	6.00	142.9	0.04
Horse 11	5.93	2.0	0.062	11.1	39.6	0.82
Horse 12	29.6	1.5	0.108	6.41	150.4	0.05
Median	31.0	1.5	0.122	5.68	97.9	0.08
Average ± SD	31.1 ± 13.9	1.5 ± 0.50	0.118 ± 0.037	5.86* ± 2.46	103.2 ± 30.3	0.16 ± 0.22

*harmonic mean; C_{max}, maximal measured serum concentration; T_{max}, time to maximal measured serum concentration; $Lambda_z$, slope of the terminal portion of the serum concentration time curve; Half-life $lambda_z$, terminal half-life; AUC_{inf}, area under the serum concentration time curve extrapolated to infinity; AUC extrap, percent of the area under the curve that is extrapolated.

describe a method that could be used to effectively regulate the use of grapiprant in racehorses. Concentrations of grapiprant (oral dose of 2 mg/kg) were above the LOQ of our analytical assay in serum for 48 hours in all horses studied and 72–96 hours in the remainder of horses. In urine, concentrations were above the LOQ for 96 hours (the last time point collected) in all horses. The analytical method described here would allow for ample regulation of this drug, based on guidelines for other NSAIDs in the United States; however, additional time points would be required to determine the time at which grapiprant concentrations are no longer detectable.

As there are no reports describing the effects of grapiprant in horses, the anti-inflammatory and analgesic concentrations have not been determined in this species. In dogs, a minimum concentration of 114–164 ng/mL is reportedly necessary to control pain.[16] Based on the concentrations achieved in the current study, if a similar efficacious dose is necessary in horses, this cannot be achieved with an oral dose of 2 mg/kg. As the drug was well tolerated in horses at a dose of 2 mg/kg, study of higher doses would be warranted.

The current study describes grapiprant concentrations and pharmacokinetics when administered orally to exercised Thoroughbred horses. Additionally, as the use of NSAIDs is commonplace in performance horses, this study describes an analytical method that can be used by regulatory labs to control the use of grapiprant in horses. As the use of this compound has not been previously described in horses, this initial study is meant to serve as foundation for future pharmacokinetic and efficacy studies in horses.

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