Title

Pharmacokinetics of grapiprant and effects on TNF-alpha concentrations following oral administration to horses

Journal

Journal of Veterinary Pharmacology and Therapeutics, 45(5)

ISSN

0140-7783

Authors

Hoffmann, Silke L

Seminoff, Kelsey

McKemie, Daniel S

et al.

Publication Date

2022-09-01

DOI

10.1111/jvp.13076

Peer reviewed

Received: 25 March 2022 | Revised: 27 April 2022 | Accepted: 19 May 2022

DOI: 10.1111/jvp.13076

JOURNAL OF Veterinary Pharmacology and Therapeutics WILEY

ORIGINAL ARTICLE

Pharmacokinetics of grapiprant and effects on TNF-alpha concentrations following oral administration to horses

Silke L. Hoffmann[1] | Kelsey Seminoff[1] | Daniel S. McKemie[1] | Philip H. Kass[2] | Heather K. Knych[1,3]

[1]K.L Maddy Equine Analytical Pharmacology Laboratory, School of Veterinary Medicine, University of California, Davis, California, USA

[2]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, California, USA

[3]Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, California, USA

Correspondence
Heather K. Knych, K.L. Maddy Equine Analytical Pharmacology Laboratory University of California, Davis, School of Veterinary Medicine, 620 West Health Science Drive, Davis, CA 95616, USA.
Email: hkknych@ucdavis.edu

Abstract

Grapiprant is a prostaglandin E_2 receptor antagonist that has been found to be an effective anti-inflammatory in dogs and that is devoid of some of the adverse effects associated with traditional NSAIDs that elicit their effects through inhibition of PGE_2 production. Previously published reports have described the pharmacokinetics of this drug in horses when administered at 2 mg/kg; however, pharmacodynamic effects in this species have yet to be described. The objective of the current study was to describe the pharmacokinetics and pharmacodynamics of grapiprant at a higher dose. Eight horses received a single oral administration of 15 mg/kg. Plasma concentrations were determined for 96 h using liquid chromatography–tandem mass spectrometry. Non-compartmental analysis was used to determine pharmacokinetic parameters. Pharmacodynamic effects were assessed ex vivo by stimulating blood samples with PGE_2 and determining TNF-α concentrations. Maximum concentration, time to maximum concentration and area under the curve were 327.5 (188.4–663.0) ng/ml, 1 (0.75–2.0) hour and 831.8 (512.6–1421.6) h*ng/ml, respectively. The terminal half-life was 11.1 (8.27–21.2) hr. Significant stimulation of TNF alpha was noted for 2–4 h post-drug administration. Results of this study suggest a short duration of EP4 receptor engagement when administered at a dose of 15 mg/kg.

KEYWORDS
grapiprant, horse, NSAID, pharmacokinetics, TNF alpha

1 | INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are arguably one of the most commonly administered classes of therapeutics in equine medicine. While there are several effective NSAIDs approved by the Food and Drug Administration for use in horses, many are associated with unwanted side effects such as gastric ulceration, inhibition of intestinal healing following ischemic injury, right dorsal colitis, renal toxicity and inhibition of bone and wound healing (Knych, 2017). These untoward effects are often attributed to inhibition of cyclooxygenase enzymes (COX), namely COX-1.

Grapiprant (Galliprant®), is a novel 'non-traditional NSAID', approved for the use in dogs (Sartini & Giorgi, 2021). In contrast to traditional NSAIDs that inhibit production of eicosanoids through COX inhibition, grapiprant blocks the binding of these mediators to receptors, specifically binding of Prostaglandin E_2 (PGE_2) to the EP_4 receptor (Sartini & Giorgi, 2021). In addition to playing a role in certain homeostatic processes, PGE_2 can also elicit inflammation,

sensitize sensory neurons and increase vasodilation and vascular permeability (Boyd et al., 2011; Chen et al., 2010; Lin et al., 2006; Nagahisa & Okumura, 2017; Nakao et al., 2007). The EP_4 receptor serves as the primary mediator of the PGE_2 mediated proinflammatory pathway responsible for these effects (Sartini & Giorgi, 2021). Since grapiprant does not interfere with the production of PGE_2, but instead acts as a receptor antagonist for the proinflammatory pathway, it presumably inhibits the inflammatory effects of PGE_2 while preserving its ability to participate in normal physiologic functions within the body (Rausch-Derra et al., 2016). Several EP4 antagonists have been described, and efficacy has been demonstrated in a variety of animal models of arthritis.

Grapiprant administration has been found to be an effective and well-tolerated method of pain management in dogs (Rausch-Derra et al., 2016) and could potentially be an effective anti-inflammatory therapeutic in horses. Currently, there are only two studies describing the administration of grapiprant to horses (Cox et al., 2020; Knych et al., 2018). Both studies are pharmacokinetic studies that assessed the absorption and disposition of grapiprant following oral administration of the label dose for dogs (2 mg/kg) (Cox et al., 2020; Knych et al., 2018). Although it is important to note that the clinical effects as well as therapeutic blood concentration for horses have yet to be determined, neither horse study (Cox et al., 2020; Knych et al., 2018) achieved concentrations reported to be anti-inflammatory in the dog (114–164 ng/ml) (Nagahisa & Okumura, 2017). However, grapiprant administration was well tolerated in both equine studies and both sets of investigators concluded that the results were encouraging for further study, including assessment of higher and/or multiple doses (Cox et al., 2020; Knych et al., 2018).

In studies conducted in humans, the effect of an EP_4 receptor antagonist on engagement of the target receptor was determined by assessing TNF-α concentrations in blood following treatment with the compound (Jin et al., 2018). The assay is based on the premise that EP_4 receptor antagonists reverse the PGE_2 inhibition of TNF-α secretion by monocytes after induction by lipopolysaccharide. Based on this, measurement of the concentration of TNF-α at multiple times post-administration, following ex vivo stimulation with LPS, can be used as a surrogate to describe the magnitude and duration of grapiprant's pharmacodynamic effect. Significantly higher concentrations of TNF-α in samples containing grapiprant compared with non-grapiprant containing samples is then suggestive of a significant pharmacodynamic effect.

In the current study, we hypothesized that oral grapiprant administration to horses, at doses higher than that studied previously, would achieve blood concentrations capable of eliciting a sustained pharmacodynamic effect with minimal side effects. To that end, the objectives of this study were to (1) to describe the pharmacokinetics of grapiprant in horses following administration of a higher dose than previously reported and (2) determine the effective plasma concentration in horses using a previously described ex vivo model of EP_4 receptor antagonism.

2 | MATERIALS AND METHODS

2.1 | Horses

Eight healthy, University-owned, exercised, Thoroughbred horses (5 mares and 3 geldings, 4–8 years of age) weighing 487–572 kg were studied. Prior to commencement of the study, horses were determined healthy based on physical examination, complete blood count and serum biochemistry panel. Blood analyses were performed by the Clinical Diagnostic Laboratories of the William R. Pritchard Veterinary Medical Teaching Hospital of the University of California, Davis, using their standard protocols. Horses did not receive any medications for a minimum of 2 weeks prior to the start of the study. The study was conducted in accordance with the Institutional Animal Care and Use Committee of the University of California, Davis.

2.2 | Study design and drug administration

Prior to drug administration, an intravenous catheter was aseptically placed in one external jugular vein for sample collection. Horses were fasted for 12 h prior to, and 4 h post-drug administration. Animals received a single oral administration, of 15 mg/kg grapiprant (Galliprant®, Elanco). Tablets were crushed into a powder and added to 60 ml of water in a dosing syringe for administration. The dosing suspension was administered within 1 h of preparation. The dose was determined using the simulation module of a pharmacokinetic modeling program (Phoenix Winnonlin, Certara), the assumption of linear pharmacokinetics, a target concentration of 114–164 ng/ml (effective concentration range in dogs (Nagahisa & Okumura, 2017) as this is as of yet unknown in the horse) and blood concentrations from a previously conducted study (Knych et al., 2018). Horses were monitored, and any visually observable adverse effects noted following each sample collection. This continued for the duration of the study.

2.3 | Sample collection

Blood samples for drug concentration determination were collected at time 0 (prior to drug administration) and at 15, 30 and 45 minutes, and 1, 2, 3, 4, 5, 6, 8, 12, 18, 24, 30, 36, 48, 72 and 96 hours following drug administration. Prior to drawing each sample of blood for analysis of drug concentrations, 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. Catheters were removed following collection of the 24-hour sample and the remaining samples collected by direct venipuncture into EDTA containing blood tubes. Samples were placed on ice until centrifugation (3000 × *g*). Following centrifugation, the plasma was immediately transferred into storage cryovials and stored at −20°C until analysis.

TABLE 1 Accuracy and precision values for LC–MS/MS analysis of grapiprant in equine plasma

Concentration (ng/mL)	Intra-day Accuracy (% nominal conc)	Intra-day Precision (% relative SD)	Inter-day Accuracy (% nominal conc)	Inter-day Precision (% relative SD)
0.03	90.0	20.0	101	17.0
10	107	10.0	104	7.0
50	113	2.0	111	4.0
500	110	6.0	106	5.0

Blood samples for the determination of TNF alpha concentrations were collected prior to drug administration and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48 and 72 h post-drug administration as described for samples collected for drug concentration determination. Samples were collected into blood tubes containing heparin and placed on ice until processed (within 1 h of collection).

2.4 | Sample analysis for determination of drug concentrations

The concentration of grapiprant was be measured in plasma by liquid chromatography tandem mass spectrometry using a previously validated method for horses (Knych et al., 2018).

2.5 | Determination of pharmacokinetic parameters

Pharmacokinetic modelling was performed using commercially available software (Phoenix WinNonlin v8.1 Certara) and non-compartmental analysis. The maximum plasma concentration (C_{max}) and time of maximum plasma concentration (T_{max}) were determined based on visual inspection of the plasma concentration data. The area under the curve was calculated using the log up-linear down trapezoidal method and extrapolation to infinity using the last measured plamsa concentration divided by the terminal slope (λ_z). The terminal phase half-life was calculated using the formula $t_{1/2} = 0.693/\lambda_z$.

2.6 | TNF alpha ex vivo assay

The effect of grapiprant on TNF-α release was determined using a previously described ex vivo model (Jin et al., 2018). Prostaglandin E_2 was added to 1 ml of whole blood (final concentration of 10 nM) and samples incubated for 30 min at 37°C and 5% CO_2. Lipopolysaccharide was then added (final concentration of 10 μg/ml) and the samples incubated for 24 hours at the conditions described above. The reaction was terminated by chilling the samples. The samples were subsequently centrifuged, and plasma collected. Concentrations of TNF-α were determined using a previously validated equine TNF-α immunoassay (R & D Systems).

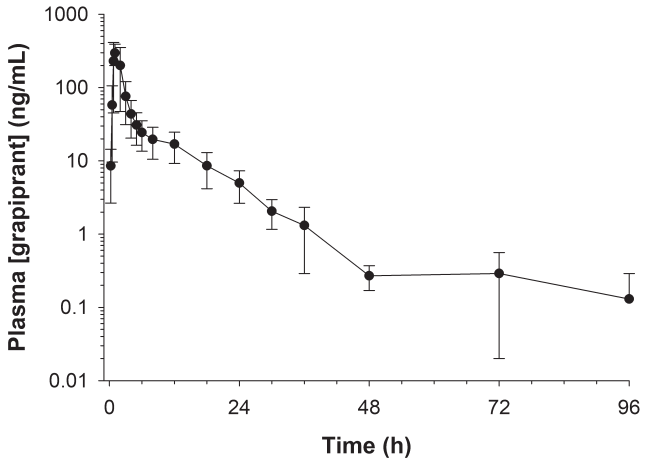

FIGURE 1 Grapiprant plasma concentrations (mean ± SD) with respect to time curve following a single oral administration of 15 mg/kg to 8 horses

2.7 | Statistical analyses

Commercially available software (Stata/BE 17.0, StataCorp) was used to assess significant differences in TNF-α concentrations between baseline and each time point. Data were analyzed using a mixed effects analysis of variance, with the individual horse as the random effect, and time as the fixed effect. Post hoc comparisons were performed with a Bonferroni multiple-comparison adjustment to preserve a nominal significance level of 0.05. Following generation of the statistical model, standardized residuals were calculated and examined for departures from normality using a normal probability plot.

3 | RESULTS

No adverse effects were noted at any time post-drug administration. The instrument response for the analytical method used to determine grapiprant concentrations was linear with a correlation coefficient of 0.99. The intra-day and inter-day precision and accuracy of the assay were determined by assaying quality control samples in replicates ($n = 6$). Accuracy was reported as percent nominal concentration and precision as percent relative standard deviation (Table 1). The technique was optimized to provide a limit of quantitation of

0.01 ng/ml and a limit of detection of approximately 0.005 ng/ml for grapiprant.

The concentrations of grapiprant at 2 h post-administration ranged from 81.1–497.1 ng/ml and at 4 h from 20.5–80.1 ng/ml. Plasma concentrations of grapiprant in the current study reached and stayed above the effective concentration for dogs (114–164 ng/ml) for 2–3 h post-administration (Figure 1). The geometric mean and range for C_{max}, T_{max} and the AUC_{inf} were 327.5 (188.4–663.0) ng/ml, 1 (0.75–2.0) hour and 831.8 (512.6–1421.6) h*ng/ml, respectively (Table 2). The terminal half-life (harmonic mean [range]) was 11.1 (8.27–21.2) hour (Table 2).

Following grapiprant administration, the concentration of TNF-α was increased 65.3% (mean) at 1 h and 47.2% at 2 h, relative to baseline, with a decrease observed in all horses at 4 h (−32.4%) (Figure 2). A significant ($p < .05$) increase in concentrations was noted at 1 and 2 h post-grapiprant administration with concentrations decreasing thereafter.

4 | DISCUSSION

The current study adds to and expands upon existing information describing the pharmacokinetics of grapiprant in horses. In agreement with previous studies in horses (Knych et al., 2018) and other species (Lebkowska-Wieruszewska et al., 2017; Łebkowska-Wieruszewska et al., 2017; Rausch-Derra et al., 2016), blood concentrations and PK parameters vary greatly between individual animals within the same study, suggesting variability with respect to absorption and distribution.

Two previously published reports describe administration of the labelled dose for dogs to horses; however, concentrations described as being therapeutic in dogs were not achieved (Cox et al., 2020; Knych et al., 2018). In the current study, a much higher dose (15 mg/kg) was administered and while concentrations deemed therapeutic for dogs were achieved, levels were only maintained for 2–3 hours post-administration. The dose for the current study (15 mg/kg) was determined using concentrations from a previously conducted study (2 mg/kg dose) (Knych et al., 2018) and the assumption of linear kinetics. Comparison of the AUC from the previously conducted study (Knych et al., 2018) and the AUC determined in the current study supports linear kinetics between doses of 2 and 15 mg/kg in horses. This is in contrast to studies in dogs, whereby a non-linear increase in AUC, and bioavailability was reported following oral administration of 1, 3 and 10 mg/kg (Nagahisa & Okumura, 2017). Similarly, in a second study in dogs, a greater than dose-dependent change in AUC was reported at doses of 1 and 50 mg/kg (Rausch-Derra et al., 2016). In both studies, investigators suggested this may be due to saturation of efflux transporters and/or metabolic enzymes.

While the AUC appeared to increase in a linear fashion between doses of 2 and 15 mg/kg, the elimination of grapiprant does not appear to be linear within the same dose range in horses. In the current study, the terminal half-life was prolonged (11.1 ± 2.71 h) compared with the previous report (5.86 ± 2.46 h), whereby the lower dose of 2 mg/kg was administered. A possible explanation for non-linear elimination in the horse is saturation of metabolic processes. Although the specific enzymes have yet to be identified, grapiprant is known to undergo hepatic metabolism in other species and therefore saturation of metabolic enzymes may also explain the increase in the terminal half-life at higher doses.

While the dose used in the current study was based on target concentrations known to be efficacious in dogs, the effective concentration for horses has yet to be determined. Therefore, a secondary goal of the current study was to determine a concentration that would lead to inhibition of the EP_4 receptor in this species. To that end, an ex vivo model of EP_4 receptor antagonism that has been utilized in previously published studies describing the target engagement of other EP_4 receptor antagonists was utilized (Jin et al., 2018; Murase et al., 2008). With this assay, an increase in TNF-α concentrations following treatment with a EP_4 receptor antagonist is indicative of inhibition of PGE_2 binding by the antagonist. Following oral administration of a 15 mg/kg dose of grapiprant, an increase in TNF-α concentrations was observed, although the duration of inhibition was short (2–4 h). Stimulation of TNF-α was observed in all animals at 2 h, corresponding to grapiprant concentrations ranging from 81.1–497.1 ng/ml. By 4 h,

TABLE 2 Pharmacokinetic parameters for grapiprant following a single oral administration of 15 mg/kg to adult horses

	Mean			
Parameter	**Geometric**	**Arithmetic**	**Median**	**Range**
C_{max} (ng/ml)	327.5	355.8	317.3	188.4–663.0
T_{max} (h)	1.10	1.22	1.0	0.75–2.0
$Lambda_z$(1/h)	0.061	0.063	0.064	0.033–0.084
HL $Lambda_z$ (h)*	---	11.0*	10.9	8.27–21.2
AUC_{inf} (h*ng/ml)	832	880	790	513–1422
AUC extrap (%)	0.16	0.21	0.14	0.04–0.54

Note: Values are reported as geometric mean and range and were generated using non-compartmental analysis.

Abbreviations: *, harmonic mean; AUC_{extrap}, extrapolated portion of the AUC; AUC_{inf}, area under the curve extrapolated to infinity; C_{max}, maximum measured concentration; HL $Lambda_z$, terminal half-life; $Lambda_z$, terminal slope; T_{max}, time of maximum concentration.

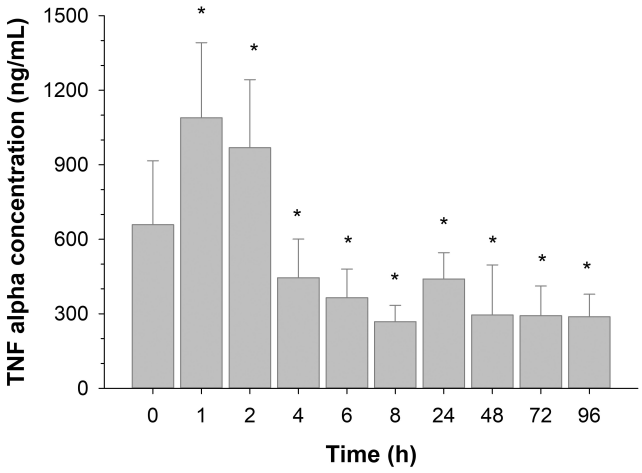

FIGURE 2 TNF-alpha concentration (mean ± SD) over time prior to and following an oral administration of 15 mg/kg grapiprant to 8 horses. *, represents a statistically significant difference in TNF alpha concentrations when compared to baseline

the point at which the TNF-α stimulation was no longer observed, grapiprant concentrations ranged from 20.5–80.1 ng/ml. Based on these findings, it is likely that grapiprant concentrations above 80 ng/ml are necessary for effective EP_4 receptor antagonism. It is important to note, however, that concentrations of TNF-α were not assessed between 2 and 4 h; so, it is not possible to ascertain if grapiprant concentrations during that period of time are capable of inhibiting PGE_2 binding to the EP_4 receptor. Unexpectedly, concentrations of TNF-α were significantly decreased, relative to pre-treatment levels, starting at 4 h post-administration. This is in contrast to a previous report, whereby TNF-α concentrations eventually declined to pre-treatment values, but were not significantly less (Jin et al., 2018). It is also important to note the large degree of variability in TNF-α concentrations at each time point. In a previously reported study, one group of investigators reported that differences in sample handling and processing as well as inter-individual subject variability could impact TNF-α concentrations (van der Linden et al., 1998). In the current study, all samples were processed immediately and handled similarly, making the most likely explanation for the large variability in TNF-α concentrations a result of subject differences with respect to production of the cytokine. Variability in TNF-α concentrations between study subjects has been described in other studies and has been suggested to be a limitation of the TNF-α ex vivo assay with respect to defining the dose–response relationship (Jin et al., 2018).

Although it is not possible to directly compare the pharmacodynamic effect in the current study (EP_4 receptor antagonism) to effects assessed in previously published reports in dogs (signs of osteoarthritis), because the clinically observable effects in dogs are presumably related to EP_4 receptor antagonism, the results presented here suggest a higher dose is necessary to achieve a therapeutic effect in horses compared with dogs. Although the 15 mg/kg dose is much higher than that reported for alleviation of clinical signs of osteoarthritis, it is lower than that reported for necessary for the treatment of inflammation in rats (Nakao et al., 2007; Okumura et al., 2008). While notably, plasma concentrations, which are important in comparing of pharmacodynamic effects, were not determined in these studies, oral doses of 19 mg/kg and 29 mg/kg (q12 hours) were found to be effective in rats with experimentally induced chronic inflammatory pain and arthritis (Nakao et al., 2007; Okumura et al., 2008).

To the best of our knowledge, there are currently no published studies correlating the EP4 receptor assay with clinical effects in any species; however, assuming a correlation, the short duration of EP_4 receptor antagonism observed in the current study is not likely to be clinically beneficial in horses. Administration of a higher dose, than that studied here will likely result in a more sustained pharmacodynamic effect, however, from a practical standpoint this would be difficult. Currently, available formulations (60 and 100 mg tablets) would require many tablets be administered which is likely not feasible, at least not suspended in water and using an oral dosing syringe. Furthermore, at this time, administration of higher doses may be cost prohibitive.

Results of the current study demonstrate that oral administration of 15 mg/kg of grapiprant could achieve and maintain concentrations above the effective concentration reported for dogs, for 2–3 h. Results of the ex vivo target activation model suggest that the minimum effective blood concentration is greater than 80 ng/ml, which correlates with significant antagonism of the EP4 receptor, as evidenced by TNF alpha stimulation, for between 2 and 4 h.

ACKNOWLEDGMENTS

This study was funded with program funds from the KL Maddy Equine Analytical Pharmacology lab. The authors would also like to acknowledge the Students Training in Advanced Research (STAR) Fellowship at the University of California, Davis School of Veterinary Medicine, for supporting Silke Hoffman during the course of this study. The authors would like to thank Stacy Steinmetz, Kirsten Kanarr, Sandy Yim and Russell Baden for technical assistance.

CONFLICTS OF INTEREST

None of the authors have any conflicts of interest to report.

AUTHOR CONTRIBUTIONS

HK contributed significantly to the conception and design of this experimental protocol. SH, HK, KS, DM and PK participated in study conduct and data analysis and interpretation. All authors edited and reviewed the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ANIMAL WELFARE STATEMENT

The authors confirm that they have adhered to US standards for the protection of animals used for scientific purposes.

ORCID

Heather K. Knych https://orcid.org/0000-0002-8676-4970

REFERENCES

Boyd, M. J., Berthelette, C., Chiasson, J.-F., Clark, P., Colucci, J., Denis, D., Han, Y., Lévesque, J.-F., Mathieu, M.-C., Stocco, R., Therien, A., Rowland, S., Wrona, M., & Xu, D. (2011). A novel series of potent and selective EP(4) receptor ligands: Facile modulation of agonism and antagonism. *Bioorganic & Medicinal Chemistry Letters*, *21*(1), 484–487. https://doi.org/10.1016/j.bmcl.2010.10.106

Chen, Q., Muramoto, K., Masaaki, N., Ding, Y., Yang, H., Mackey, M., Li, W., Inoue, Y., Ackermann, K., Shirota, H., Matsumoto, I., Spyvee, M., Schiller, S., Sumida, T., Gusovsky, F., & Lamphier, M. (2010). A novel antagonist of the prostaglandin E(2) EP(4) receptor inhibits Th1 differentiation and Th17 expansion and is orally active in arthritis models. *British Journal of Pharmacology*, *160*(2), 292–310. https://doi.org/10.1111/j.1476-5381.2010.00647.x

Cox, S., Sommardahl, C., Fortner, C., Davis, R., Bergman, J., & Doherty, T. (2020). Determination of grapiprant plasma and urine concentrations in horses. *Veterinary Anaesthesia and Analgesia*, *47*(5), 705–709. https://doi.org/10.1016/j.vaa.2020.04.006

Jin, Y., Smith, C., Hu, L., Coutant, D. E., Whitehurst, K., Phipps, K., McNearney, T. A., Yang, X., Ackermann, B., Pottanat, T., & Landschulz, W. (2018). LY3127760, a selective prostaglandin E4 (EP4) receptor antagonist, and celecoxib: A comparison of pharmacological profiles. *Clinical and Translational Science*, *11*(1), 46–53. https://doi.org/10.1111/cts.12497

Knych, H. K. (2017). Nonsteroidal anti-inflammatory drug use in horses. *The Veterinary Clinics of North America. Equine Practice*, *33*(1), 1–15. https://doi.org/10.1016/j.cveq.2016.11.001

Knych, H. K., Seminoff, K., & McKemie, D. S. (2018). Detection and pharmacokinetics of grapiprant following oral administration to exercised thoroughbred horses. *Drug Testing and Analysis*, *10*(8), 1237–1243. https://doi.org/10.1002/dta.2378

Łebkowska-Wieruszewska, B., Barsotti, G., Lisowski, A., Gazzano, A., Owen, H., & Giorgi, M. (2017). Pharmacokinetics and estimated bioavailability of grapiprant, a novel selective prostaglandin E2 receptor antagonist, after oral administration in fasted and fed dogs. *New Zealand Veterinary Journal*, *65*(1), 19–23. https://doi.org/10.1080/00480169.2016.1241727

Lebkowska-Wieruszewska, B., De Vito, V., Owen, H., Poapholatep, A., & Giorgi, M. (2017). Pharmacokinetics of grapiprant, a selective EP4 prostaglandin PGE2 receptor antagonist, after 2 mg/kg oral and i.v. administrations in cats. *Journal of Veterinary Pharmacology and Therapeutics*, *40*(6), e11–e15. https://doi.org/10.1111/jvp.12414

Lin, C.-R., Amaya, F., Barrett, L., Wang, H., Takada, J., Samad, T. A., & Woolf, C. J. (2006). Prostaglandin E2 receptor EP4 contributes to inflammatory pain hypersensitivity. *The Journal of Pharmacology and Experimental Therapeutics*, *319*(3), 1096–1103. https://doi.org/10.1124/jpet.106.105569

Murase, A., Taniguchi, Y., Tonai-Kachi, H., Nakao, K., & Takada, J. (2008). In vitro pharmacological characterization of CJ-042794, a novel, potent, and selective prostaglandin EP(4) receptor antagonist. *Life Sciences*, *82*(3–4), 226–232. https://doi.org/10.1016/j.lfs.2007.11.002

Nagahisa, A., & Okumura, T. (2017). Pharmacology of grapiprant, a novel EP4 antagonist: Receptor binding, efficacy in a rodent postoperative pain model, and a dose estimation for controlling pain in dogs. *Journal of Veterinary Pharmacology and Therapeutics*, *40*(3), 285–292. https://doi.org/10.1111/jvp.12349

Nakao, K., Murase, A., Ohshiro, H., Okumura, T., Taniguchi, K., Murata, Y., Masuda, M., Kato, T., Okumura, Y., & Takada, J. (2007). CJ-023,423, a novel, potent and selective prostaglandin EP4 receptor antagonist with antihyperalgesic properties. *The Journal of Pharmacology and Experimental Therapeutics*, *322*(2), 686–694. https://doi.org/10.1124/jpet.107.122010

Okumura, T., Murata, Y., Taniguchi, K., Murase, A., & Nii, A. (2008). Effects of the selective EP4 antagonist, CJ-023,423 on chronic inflammation and bone destruction in rat adjuvant-induced arthritis. *The Journal of Pharmacy and Pharmacology*, *60*(6), 723–730. https://doi.org/10.1211/jpp.60.6.0007

Rausch-Derra, L., Huebner, M., Wofford, J., & Rhodes, L. (2016). A prospective, randomized, masked, placebo-controlled multisite clinical study of Grapiprant, an EP4 prostaglandin receptor antagonist (PRA), in dogs with osteoarthritis. *Journal of Veterinary Internal Medicine*, *30*(3), 756–763. https://doi.org/10.1111/jvim.13948

Sartini, I., & Giorgi, M. (2021). Grapiprant: A snapshot of the current knowledge. *Journal of Veterinary Pharmacology and Therapeutics*, *44*, jvp.12983. https://doi.org/10.1111/jvp.12983

van der Linden, M. W., Huizinga, T. W., Stoeken, D. J., Sturk, A., & Westendorp, R. G. (1998). Determination of tumour necrosis factor-alpha and interleukin-10 production in a whole blood stimulation system: Assessment of laboratory error and individual variation. *Journal of Immunological Methods*, *218*(1–2), 63–71. https://doi.org/10.1016/s0022-1759(98)00108-2

How to cite this article: Hoffmann, S. L., Seminoff, K., McKemie, D. S., Kass, P. H., & Knych, H. K. (2022). Pharmacokinetics of grapiprant and effects on TNF-alpha concentrations following oral administration to horses. *Journal of Veterinary Pharmacology and Therapeutics*, *45*, 467–472. https://doi.org/10.1111/jvp.13076