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Tolerance and Pharmacokinetics of Galliprant™ Administered Orally to Collies Homozygous for MDR1-1Δ

Mark C. Heit[1] | **Katrina L. Mealey**[2] | **Stephen B. King**[1]

[1]Elanco Animal Health, Inc, Greenfield, IN, USA

[2]Progam in Individualized Medicine (PrIMe), College of Veterinary Medicine, Washington State University, Pullman, WA, USA

Correspondence
Mark C. Heit, Elanco Animal Health, Inc., 2500 Innovation Way, Greenfield, IN, USA.
Email: mark.heit@elancoah.com

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Abstract

The objectives of the study were to evaluate the pharmacokinetics and tolerance of grapiprant, a substrate of the human P-gp transporter, in collies homozygous for MDR1-1Δ when administered at the labeled dosage of 2 mg/kg once daily for 28 days. Twelve collie dogs with homozygous for MDR1-1Δ genotype from a commercial colony were used in the study, eight in the treated group and four as placebo-treated controls. The only treatment-related clinical sign was self-limiting vomiting (in 2/8 treated animals) and the only treatment-related clinical pathological changes seen were a slight decrease in serum albumin in one dog (2.6 g/dL; reference 2.7 to 3.9 g/dL) and total protein (5.1 g/dL; reference 5.5 to 7.7 g/dL). Absorption of grapiprant was rapid with a median T_{max} of 1 h, C_{max} of 5.2 μg/mL, $AUC_{0\text{-}24}$ of 17.3 ± 7.1 h*μg/mL and median terminal t½ of 4.3 h after the first dose. To determine whether MDR1-1Δ animals handle grapiprant differently from normal dogs, a population pharmacokinetic analysis was performed utilizing data from the collies and historical beagle data. Volume of the peripheral compartment of collies was estimated to be 45% that of beagles, and clearance from the central compartment was 71% less in collies than in beagles. Self-liming vomiting events occurred at a numerically higher rate (2/8; 25%) in this group of P-gp-deficient dogs than seen in a clinical study (17%) composed of various dog breeds but limited numbers in this PK study make comparisons difficult. Grapiprant was otherwise well tolerated in collies homozygous for MDR1-1Δ despite increased drug exposure compared to dogs without this mutation.

KEYWORDS
dog, grapiprant, MDR1, pharmacokinetics, tolerance

1 | INTRODUCTION

Grapiprant, the active ingredient in Galliprant™, is an analgesic and anti-inflammatory molecule of the piprant class that functions as a selective antagonist of the prostaglandin E_2 (PGE_2) receptor 4 (EP4 receptor) (Giorgi, 2015; Shaw et al., 2016). PGE_2 is synthesized from arachidonic acid by cyclooxygenase and specific prostanoid synthases in fibroblasts, monocytes, and epithelial and endothelial cells. PGE_2 exerts its effects via four receptors: EP1, EP2, EP3, and EP4. Unlike non-steroidal anti-inflammatory drugs that inhibit the cyclooxygenase enzymes, grapiprant does not affect the production of prostanoids. Additionally, because grapiprant

selectively antagonizes the EP4 receptor, physiologic activity of PGE_2 at the remaining three receptors is retained (Nakao et al., 2007). The result of this mechanism of action and selectivity is efficacy against PGE_2-mediated pain and inflammation while maintaining an acceptable safety profile by not affecting the production of prostanoids.

Galliprant was approved in March 2016 in the United States by the Food and Drug Administration's Center for Veterinary Medicine (FDA CVM) for the control of pain and inflammation associated with osteoarthritis in dogs (NADA 141-455, 2016). It is available in 20, 60, and 100 mg oral scored tablet presentations. It was approved in Europe by the European Medicines Agency (EMA) in September 2018 for the treatment of pain associated with mild to moderate osteoarthritis in dogs (European Medcines Agency, 2020). During product development, it was determined that grapiprant is a substrate for human P-glycoprotein (P-gp), the ABCB1 (MDR1) gene product. Because P-gp substrate drugs have been reported to have altered disposition in dogs with deficient P-gp function, it seemed prudent to investigate the disposition of grapiprant in dogs homozygous for MDR1-1Δ (MDR1 mutant/mutant). Specifically, increased brain penetration (Deshpande et al., 2016; Mealey et al., 2001; Mealey, Greene, et al., 2008) and defective biliary excretion (Coelho et al., 2009; Mealey, Fidel, et al., 2008) of P-gp substrates such as ivermectin, loperamide, technetium 99-m, acepromazine, and others have been documented in dogs homozygous for MDR1-1Δ resulting in increased susceptibility to adverse effects compared to dogs with normal P-gp function. Additionally, since P-gp is expressed by enterocytes within the gastrointestinal tract, and by renal tubular cells (Ginn, 1996), it is also possible that oral bioavailability and renal excretion of P-gp substrates could differ in dogs homozygous for MDR1-1Δ, as compared to wild-type dogs.

Thus, the objectives of this study were to evaluate the pharmacokinetics and tolerance of grapiprant in collies homozygous for MDR1-1Δ when administered at approximately the label dosage of 2 mg/kg once daily for 28 days.

2 | MATERIALS AND METHODS

2.1 | Study Design and Standards

This study was a blinded, randomized complete block parallel design with a 2:1 allotment of treated to control animals.

The study was conducted and documented in accordance with VICH GL9 Good Clinical Practice (FDA CVM, 2001) with the exception of the bioanalytical, clinical pathological, and pharmacokinetic analyses. There was no impact of these exceptions on the integrity of the data or the ability to interpret the results. Although no formal power calculations were conducted, this study was designed to use the fewest number of animals possible to provide numerically meaningful data consistent with its objectives and scientific needs. This manuscript was prepared in compliance with the ARRIVE guidelines for reporting animal in vivo experiments (Kilkenny et al., 2010).

2.2 | Animals

Twelve dogs (5 male and 7 female) considered healthy based on a pre-study physical and laboratory examinations were selected from 14 P-gp-deficient colony collie dogs owned by Cheri Hill Kennel & Supply, Inc. (Stanwood, MI, USA). Animals were not exposed within 30 days prior to a seven-day acclimation period to any oral anthelmintics or endectocides, heartworm preventives, or ear mite treatments that may have impacted the interpretation of the study results. MDR1 genotyping was performed by the Veterinary Clinical Pharmacology Laboratory (VCPL), Washington State University College of Veterinary Medicine. Dogs were uniquely identified (i.e., via USDA number and microchip). All activities involving animals were approved by the study site and Elanco IACUCs. No animals were prematurely removed from the study, and after completing the study, all were returned to the site colony.

Dog management and housing conformed to the appropriate dog care standards and relevant laboratory SOPs. Dogs were housed individually in suspended chain link cages (4′ W x 4′ L x 40″ H) with flattened uncoated wire grid bottom floors with a solid and cleanable surface pad on which the dog could lay. Animals had visual and auditory contact with conspecifics as well as surface/tactile contact with dogs in adjacent pens. Provision of food and water was consistent with the facility standards and relevant laboratory SOPs. Food was removed late in the afternoon (approximately 4 PM) prior to each day of dosing and was returned at least 1.5 h after dose administration at approximately 11 AM.

2.3 | Randomization and Masking Procedures

Dogs were assigned to treatment according to a randomized block design. The blocking factors were gender and body weight. Within each gender, animals were ordered by Day −1 body weight and blocks of three animals (i.e., three heaviest in first block) were formed. Two full blocks of three females each were formed, but because only five male animals were eligible for the study, only one full block of male animals was formed with the remaining two male animals left to fill a mixed block (i.e., 2 males and 1 female). One animal from each block was randomly assigned to the control group with the other two animals randomly assigned to the grapiprant treatment group. Study masking was achieved through separation of activities. Study site personnel who made observations were masked to the treatment group and did not participate in treatment administration to ensure unbiased observations. To maintain masking, three treatment codes were utilized (e.g., A, B, and C), two of which identified groups of 4 animals receiving grapiprant and the third being the group of 4 animals receiving the control (empty gelatin capsules).

2.4 | Treatment and Administration

Dogs were dosed orally once daily with approximately 2 mg/kg of grapiprant (Galliprant, 20 or 60 mg tablets, Elanco Animal Health) or a single empty gelatin capsule for 28 days beginning on Day 0. The target dosage per the label is 2 mg/kg; however, due to the commercially available tablet sizes and body weight bands, clinically, an animal may receive from 1.5 to 2.9 mg/kg. To better assess tolerability, body weight ranges were adjusted to ensure that dogs could receive the maximum labeled dosage and a higher minimum dosage (2.0 mg/kg). To allow for more precise dosing (as preferred in a pharmacokinetic study), smaller tablet increments and narrower dose bands were also utilized. Lastly, although not required per label, because exposure is greater in the fasted state compared to fed state, all animals were fasted overnight prior to each dose administration. Doses were calculated on the first day of dose administration (Day 0) and administered for 14 consecutive days and then recalculated on Day 14 and administered until study completion.

The tablet or capsule was administered by placing it in the back of the animal's mouth and allowing the animal to swallow it normally. Approximately 5 mL of water was administered via syringe to each animal following each dose to assist in swallowing. Animals were examined for any signs of spitting out, regurgitation, or vomiting of the tablet for at least 15 minutes following dose administration.

2.5 | Experimental Procedures

General health observations were conducted twice daily at least four hours apart. On days that physical examinations or detailed clinical observations were conducted, general health observations occurred once. Detailed clinical observations were performed weekly. Each animal was brought to the pen opening to allow palpation/manipulation and observation/evaluation of, respiration, the eyes and nose, locomotion, behavior, appetite, feces, and evidence of vomiting. Physical examinations were conducted by a veterinarian on Day −7, prior to randomization on Day −1, approximately 2 h after dose administration on Day 0 and on Day 28. Observations identified during detailed clinical observations or physical examinations were followed until resolved or until the end of the study. Because Galliprant is an approved Elanco product, all abnormal observations (including abnormal clinical pathological results) were documented and summarized as adverse events (AEs) and reported to Elanco Pharmacovigilance.

Body weights measured on Days −1 and 13 were used to determine dose amounts that were administered beginning on Days 0 and 14, respectively, and were both compared to the post-treatment body weight measured on Day 28.

Blood samples for hematology and chemistry analysis, urine samples for urinalysis, and fecal samples for fecal analysis were obtained on Day −7. Blood samples for hematology and chemistry analysis and urine samples for urinalysis were obtained on Day 28. A free catch urine collection system was implemented utilizing plastic sheeting below the cages where pools of urine were collected via syringe and transferred to a collection container. Samples were processed/analyzed by IDEXX BioResearch (North Grafton, MA, USA) except for fecal parasites, which was performed by the study site.

2.6 | Blood Sampling for Measuring Grapiprant Concentration

Blood was collected from the jugular vein pre-dose and 0.5, 1, 2, 4, 8, 12, and 24 h after the first dose from animals in the grapiprant group. Samples were obtained at these same time points for the last dose with additional samples at 34 and 48 h post-dose. Predose and 2-h samples were obtained on Days 0 and 27 from dogs in the control group to confirm lack of exposure. Samples were collected into chilled tubes containing lithium heparin anticoagulant, inverted several times, and centrifuged within 60 min of collection. All samples were maintained chilled throughout processing. The plasma from each sample was divided into two approximately equal aliquots, transferred into two labeled polypropylene microtubes and frozen at ≤ −20°C within 90 minutes of collection. All samples were kept frozen until shipped on dry ice at the conclusion of the study for bioanalysis.

2.7 | Bioanalytical Method

Samples were analyzed using a qualified LC/MS/MS method (Charles River, Tranent, Edinburgh, UK). Calibration standards and quality control samples were prepared by aliquoting 10 µL of dog plasma (K2EDTA) containing a range of 5.00–5000 and 15.0, 200 and 4000 ng/mL of grapiprant, respectively, into a 96 round well plate. Because these samples were prepared in K2EDTA dog plasma but the study samples were collected using lithium heparin, a separate qualification study was conducted which confirmed that dog plasma samples collected in lithium heparin were accurately quantified using a calibration line prepared using K2EDTA dog plasma (data not shown).

Ten microliters of internal standard (d5-grapiprant) was added to plasma samples (10 µL water/methanol (95/5, v/v) to the blanks) followed by 1 mL of 2% ammonia solution. The plate was capped, vortex mixed, and centrifuged at 2400 x g for 5 minutes at a temperature set to maintain 4°C. A Waters Oasis HLB 30 µm 10 mg 96 well SPE plate was conditioned with 500 µL of methanol followed by 500 µL of water. Using a Tomtec Quadra 4, the samples were loaded onto the SPE plate and pulled through under light vacuum. The SPE plate was washed with 5% methanol and left to dry. Once dry, the samples were eluted into a clean 96 round well plate using 250 µL of acetonitrile. The elution step was undertaken twice. The eluents were evaporated to dryness using nitrogen at a temperature of 40°C

before reconstituting in 500 µL of water/acetonitrile (90/10, v/v). The collection plate was capped, vortex mixed, and centrifuged at 2400 x g for 5 minutes at a temperature set to maintain 4°C.

Ten µL of sample was auto-injected into a Waters Acquity I-class system containing a Waters X-Bridge C_{18}, 50 x 2.1 mm, 3.5 µm column. A gradient mobile phase was used where mobile phase A was acetonitrile/methanol (70/30, v/v) and mobile phase B was water/formic acid (100/0.1, v/v). Detection was accomplished using a Sciex API6500 mass spectrometer in TurboIonSpray ionization mode where the m/z for grapiprant was 492.2 and for the internal standard was 497.2. The retention time for grapiprant was approximately 2.2 min. The lower limit of quantification was 5.00 ng/mL.

2.8 | Statistical Methods

The unit of observation and statistical analysis was the individual dog. All dogs were included in the analyses. All calculations of descriptive statistics were performed using SAS® v9.4, SAS Institute, Cary, NC.

All abnormal clinical signs observed (e.g., clinical and general health observations, physical examination results) were reported as AEs, and the number of animals experiencing the AE and animal rate were summarized by treatment group. Total incidents and incidence rates by treatment group were summarized as well. Descriptive summary statistics were calculated by treatment group for numerical data (e.g., body weights, clinical pathology, body temperature).

2.9 | Pharmacokinetic Analysis

The bioanalytical data were processed using a validated pharmacokinetic software (Phoenix, version 8.1; Pharsight Corporation, Mountain View, CA, USA). A non-compartmental pharmacokinetic analysis (Phoenix WinNonlin) was performed to determine the peak plasma concentration (C_{max}), time achieved (T_{max}), the half-life ($t_{1/2}$), and the area under the plasma concentration curve (AUC). Concentrations identified as being less than the lower limit of quantification (<LLOQ) occurring prior to T_{max} were considered 0 and were considered missing thereafter. Nominal time points were used for plotting and, when within the protocol-specified window, were used for calculations. On Day 28, seven of the eight 24-hour time point blood samples were obtained outside the 20-minute allowable window. Even though the maximum deviation was only 4 minutes outside the window, the actual time point was used in the pharmacokinetic analyses. To more accurately reflect the dosage received for the last dose (Dose 28), the body weight measured on the day after dosing (Day 28) was used in the PK analysis rather than that measured on Day 13. AUC was calculated via the "Linear Up Log Down" calculation method and the time points used in calculating the terminal slopes were selected using the "Best Fit" fit method.

Accumulation ratios were calculated for each animal according to the following formulae:

RC_{max} = C_{max} of 28th administration / C_{max} of 1st administration

R_{AUC} = $AUC_{0\text{-}24h}$ of 28th administration / $AUC_{0\text{-}24h}$ of 1st administration

The data generated in this study were analyzed in combination with historical data collected in beagle dogs during the development of the product using population pharmacokinetics (Phoenix NLME). A model was created in which the combined data (including the first and last collie dose) were best fit by a two compartmental model using proportional weighting. Covariate analysis was used to identify factors that influenced the PK of grapiprant in the sample population.

3 | RESULTS

3.1 | Dose Administered

Animals in the grapiprant group received an average dosage of 2.55 mg/kg (range: 2.12 – 2.88 mg/kg) on Days 0 – 13 and 2.54 mg/kg (range: 1.99 – 3.01 mg/kg) from Day 14 to Day 27. On three occasions (one 20 mg tablet and two empty capsules), the dose was spit out and immediately re-administered. There were no instances of post-dose vomiting observed.

3.2 | Tolerability

All enrolled dogs were healthy based on pretreatment physical examination and clinical pathological evaluation. Their average age was 4.9 years (range: 1.6 – 8.5 y). The average age of dogs in the treated and placebo groups was 4.5 y (range: 1.6 – 6.9) and 5.6 y (range: 1.6 – 8.5), respectively. Two male and two female dogs were randomized to the control group; five female and three male dogs were randomized to the grapiprant group. The average weight of dogs in both groups was ~25 kg. Body weights remained relatively consistent over the course of the study. On average, dogs in both groups lost less than 1 kg and individually, the most any dog lost was 1.05 and 1.65 kg in the control and grapiprant groups, respectively. No dogs gained weight. There were no differences in body weight nor body weight change between the two groups.

Two animals had minor skin lesions prior to the first dose administration that did not worsen after grapiprant administration. There were two minor clinical abnormalities observed during the study period. One animal in the grapiprant group became lame caused by an abscess that required treatment with hydrogen peroxide soaks and oral cefpodoxime, and one animal in the control group developed dermatitis on the dorsum of its neck that required no therapy. There were no treatment-related effects on quantitative parameters of the physical examination.

The only clinical pathological changes seen, both considered to be related to treatment with grapiprant, were mild hypoalbuminemia in one male dog (pretreatment =3.2 g/dL, Day 28 = 2.6 g/dL;

TABLE 1 Summary statistics (mean ±SD) for continuous parameters of clinical pathological and physical examinations

			Control		Grapiprant	
	Units	Normal Range	Pre-treat	Post-treat	Pre-treat	Post-treat
ALB/GLOB Ratio	N/A	N/A	1.1 ± 0.14	1.13 ± 0.15	1.08 ± 0.19	1.1 ± 0.24
ALP	U/L	(5, 160)	14.5 ± 5.8	18.75 ± 6.75	12 ± 5.55	18 ± 8.09
ALT	U/L	(18, 121)	34.5 ± 6.4	31.25 ± 6.6	29.75 ± 5.26	24.75 ± 3.37
AST	U/L	(16, 55)	22.25 ± 2.5	21 ± 4.97	22.75 ± 7.32	18.88 ± 3.64
Albumin	g/dL	(2.7, 3.9)	3.4 ± 0.18	3.38 ± 0.22	3.4 ± 0.16	3.15 ± 0.36
BUN	mg/dL	(9, 31)	17.75 ± 3.1	18 ± 1.41	16 ± 4.14	18.13 ± 4.52
BUN/Creat Ratio	N/A	N/A	18.3 ± 3.57	19.23 ± 1.95	17.44 ± 5.56	19.09 ± 3.56
Bicarbonate TCO2	mmol/L	(13, 27)	17.25 ± 1.71	17.5 ± 1.91	18.5 ± 1.07	18.88 ± 0.83
Bilirubin; Conj	mg/dL	(0, 0.1)	0.08 ± 0.05	0 ± 0	0.08 ± 0.05	0 ± 0
Bilirubin; Unconj	mg/dL	(0, 0.2)	0.2 ± 0	0.3 ± 0	0.19 ± 0.06	0.25 ± 0.05
Calcium	mg/dL	(8.8, 11.2)	9.8 ± 0.34	9.85 ± 0.19	9.93 ± 0.31	9.76 ± 0.44
Chloride	mmol/L	(108, 119)	113.25 ± 2.63	113.75 ± 2.22	111.88 ± 1.46	113.88 ± 1.13
Cholesterol	mg/dL	(131, 345)	340.25 ± 59.9	346.75 ± 52.47	299.13 ± 57.61	293.38 ± 58.33
Creatine Kinase	U/L	(10, 200)	74.75 ± 15.97	72 ± 26.09	140.5 ± 194.21	67.38 ± 26.03
Creatinine	mg/dL	(0.5, 1.5)	1 ± 0.28	0.95 ± 0.17	0.94 ± 0.13	0.95 ± 0.13
Globulin	g/dL	(2.4, 4)	3.13 ± 0.25	2.97 ± 0.17	3.24 ± 0.55	2.99 ± 0.54
Glucose	mg/dL	(63, 114)	103.25 ± 5.97	104 ± 5.94	101.13 ± 4.45	107 ± 6.16
Phosphorus	mg/dL	(2.5, 6.1)	2.4 ± 0.45	3.55 ± 0.66	2.81 ± 0.64	3.83 ± 0.65
Potassium	mmol/L	(4, 5.4)	4.1 ± 0.08	4.2 ± 0.18	4.09 ± 0.16	4.29 ± 0.25
Sodium	mmol/L	(142, 152)	144.25 ± 1.26	146 ± 1.41	144.38 ± 1.6	145.63 ± 1.6
Na/K Ratio	N/A	N/A	35.25 ± 0.96	34.75 ± 1.5	35.5 ± 1.41	34 ± 2.2
Total Bilirubin	mg/dL	(0, 0.3)	0.28 ± 0.05	0.3 ± 0	0.26 ± 0.05	0.25 ± 0.05
Total Protein	g/dL	(5.5, 7.5)	6.53 ± 0.15	6.35 ± 0.19	6.64 ± 0.51	6.14 ± 0.48
Fibrinogen	mg/dL	(90, 255)	NS	173.75 ± 21.31	NS	181 ± 68.01
PTT	sec	(10.6, 16.8)	NS	11.58 ± 1.12	NS	11.35 ± 1.19
Prothrombin Time	sec	(6.3, 13.3)	NS	7.85 ± 0.13	NS	8.54 ± 1.69
Basophils Abs	/uL	(0, 100)	5.25 ± 6.4	6.75 ± 5.32	1.38 ± 3.89	3 ± 4.17
Eosinophils Abs	/uL	(70, 1490)	672.25 ± 509.33	540.5 ± 189.51	654.38 ± 393.18	734.88 ± 367.35
Lymphocytes Abs	/uL	N/A	1487 ± 138.54	1528.5 ± 147.16	1549.1 ± 415.3	1539.8 ± 341.5
Monocytes Abs	/uL	(130, 1150)	494.5 ± 53.71	485 ± 138.35	468.38 ± 187.13	452.13 ± 144.02
Neutrophil Abs	/uL	(2940, 12670)	4191.5 ± 867.95	4389.8 ± 385.17	5101.8 ± 1687.2	4733.4 ± 890.71
HCT	%	(38.3, 56.5)	48.45 ± 2.54	49.98 ± 5.65	47.91 ± 2.75	46.91 ± 4.86
HGB	g/dL	(13.4, 20.7)	16.75 ± 0.81	17.3 ± 2.24	16.39 ± 1.17	15.95 ± 1.93
MCH	pg	(21.9, 26.1)	22.48 ± 0.67	22.55 ± 0.57	22.31 ± 0.49	22.61 ± 0.57
MCHC	g/dL	(32.6, 39.2)	34.58 ± 0.53	34.58 ± 0.84	34.18 ± 0.57	33.94 ± 0.83
MCV	fL	(59, 76)	65 ± 2.16	65.25 ± 1.26	65.13 ± 1.64	66.75 ± 2.31
RBC	M/uL	(5.39, 8.7)	7.45 ± 0.15	7.65 ± 0.81	7.35 ± 0.53	7.06 ± 0.9
WBC	K/uL	(4.9, 17.6)	6.85 ± 0.57	6.95 ± 0.35	7.78 ± 2.03	7.46 ± 1.44
Basophils	%	N/A	0.08 ± 0.1	0.1 ± 0.08	0.01 ± 0.04	0.04 ± 0.05
Eosinophils	%	N/A	10.05 ± 8.05	7.83 ± 2.97	8.83 ± 6.03	9.49 ± 3.77
Lymphocytes	%	N/A	21.75 ± 1.89	21.98 ± 1.45	20.41 ± 4.34	20.73 ± 2.67
Monocytes	%	N/A	7.28 ± 1.16	6.95 ± 1.82	5.91 ± 1.18	6.09 ± 1.55
Neutrophil	%	N/A	60.85 ± 8.87	63.15 ± 4.18	64.84 ± 6.09	63.66 ± 4.59
Specific Gravity	N/A	N/A	1.06 ± 0.02	1.06 ± 0.01	1.04 ± 0.02	1.06 ± 0.02

(Continues)

TABLE 1 (Continued)

	Units	Normal Range	Control		Grapiprant	
			Pre-treat	Post-treat	Pre-treat	Post-treat
pH	N/A	N/A	7.63 ± 0.85	6.5 ± 0.41	7.75 ± 0.71	6.94 ± 1.02
Cardio Rate	beats/min	N/A	104 ± 22.45	105 ± 10	104 ± 13.44	110 ± 11.95
Respiration	breaths/min	N/A	82.5 ± 9.57	56 ± 32.82	78.75 ± 14.58	56.75 ± 30.96
Temperature	◦C	N/A	101.5 ± 1.02	101.7 ± 1.49	101.2 ± 0.63	101.6 ± 1.26

TABLE 2 Adverse event incidence rates (by number of animals and total incidents) in MDR1-1 Δ collies administered placebo or grapiprant at 2 mg/kg once daily for 28 days

Preferred Term	Grapiprant (N=8)		Control (N=4)	
	Number of Animals (%)	Number of Incidents (Incidence Rate*)	Number of Animals (%)	Number of Incidents (Incidence Rate*)
Dermatitis and eczema	0	0	1 (25.0%)	1 (0.0089)
Emesis	2 (25.0%)	4 (0.0179)	0	0
Lameness	1 (12.5%)	1 (0.0045)	0	0
Bacterial skin infection	1 (12.5%)	1 (0.0045)	0	0
Trauma NOS	1 (12.5%)	1 (0.0045)	0	0

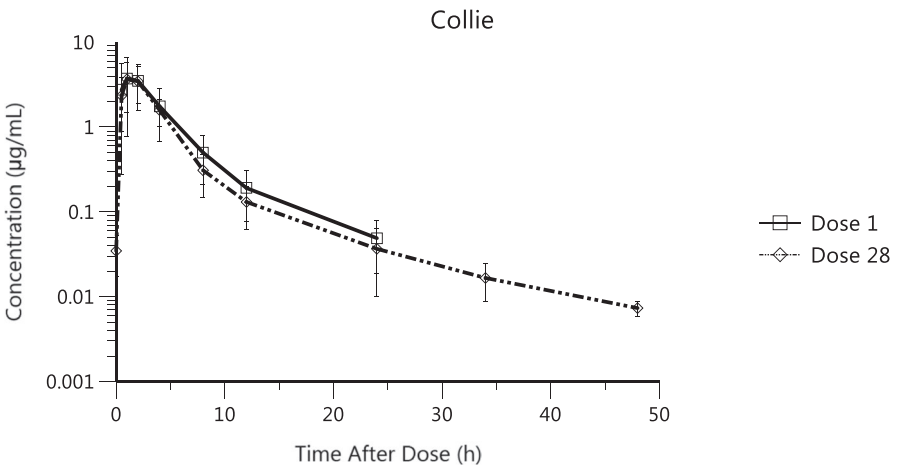

FIGURE 1 Mean (arithmetic) profile plots ±standard deviation after the first (Dose 1) and last dose (Dose 28) of grapiprant administered to MDR1-1Δ collies (N=8) at 2 mg/kg once daily for 28 days. Seven and four dogs had measurable concentrations at 34 and 48 h, respectively

reference 2.7 to 3.9 g/dL) and mild hypoproteinemia in one female dog (pretreatment =6.0 g/dL, Day 28 = 5.1 g/dL; reference 5.5 to 7.7 g/dL). Mean values of both parameters for all treated dogs remained within the normal range at all time points. No other changes were observed in the clinical pathological or physical examinations (Table 1).

There were very few adverse events of which none were serious (Table 2). A skin lesion developed and progressed to an abscess in one dog from the grapiprant-treated group. Because this abscess involved the interdigital footpad, it was considered to be traumatic in origin rather than related to the treatment. Vomiting (emesis) as evidenced by vomitus under the cage (i.e., the animal was not observed vomiting) occurred in 2/8 treated and 0/4 control animals for a total of four self-limiting events. It occurred a single time in one animal and on 3 separate days (at least 3 days apart) in the second. Because it was observed only in treated animals, it was attributed to the treatment.

3.3 | Pharmacokinetic Analysis

The analytical batches met acceptance criteria. A calibration curve from 5 to 5000 ng/mL was achieved with %CV ranging from 6.6 to 17.7%. All quality control samples fell within ±30% of the nominal concentration. The LLOQ was 5.00 ng/mL.

Pre-values (i.e., prior to the first dose) in both groups were below the LLOQ. Grapiprant was detected from all grapiprant-treated dogs after dose administration and from no dogs treated with placebo. Three of the eight grapiprant-treated dogs had measurable concentrations 48 h after the last dose, and all but one had measurable

TABLE 3 Pharmacokinetic parameters measured after the first and last dose of grapiprant administered to eight MDR1-1 Δ collies at 2 mg/kg once daily for 28 days

	C_{max} (μg/mL)	T_{max} (h)	$t_{1/2}$† (h)	AUC‡ (h*μg/mL)	Vz/F (mL/kg)	Cl/F (mL/h/kg)
Dose 1	5.2 ± 2.0	1.2 ± 0.7	4.3 (3.4–6.0)	17.3 ± 7.1	1110 ± 552	169 ± 72.3
Dose 28	4.6 ± 2.0	1.3 ± 0.6	7.1 (4.5–8.9)	15.2 ± 4.5	1830 ± 861	182 ± 83.0

†Data presented as mean ±SD except for $t_{1/2}$ which is median (range).
‡AUC from 0 to 24 h after the dose.

TABLE 4 Model estimates resulting from population pharmacokinetic analysis of eight MDR1-1 Δ collies that received oral 2 mg/kg grapiprant daily for 28 days and from 16 beagle dogs that received a single oral dose of grapiprant at 6 mg/kg

Parameter	Estimate	SD	CV%	Units
tvKa	0.59	0.03	5.18	1/h
tvV	270.43	45.39	16.79	mL/kg
tvV2	1243.75	296.87	23.87	mL/kg
tvCl	393.66	31.22	7.93	mL/(kg*h)
tvCl2	63.77	9.28	14.56	mL/(kg*h)
dCldBreed0	−0.79	0.12	−14.70	
dV2dBreed0	−1.25	0.21	−16.56	
stdev0	0.52	0.05	8.82	

concentrations in the 34 h sample. After the first and last doses, absorption appeared to be rapid with average maximum concentrations of approximately 5.2 μg/mL and 4.6 μg/mL, respectively, occurring with a median T_{max} of 1 h after dose administration (Figure 1). AUC measured from 0 to 24 h after the first and last dose was estimated to be on average (±SD) 17.3 ± 7.1 h*μg/mL and 15.2 ± 4.5 h*μg/mL, respectively (Table 3). Median half-life after the first dose was less than that after the last dose (4.3 and 7.1 h, respectively). The variability of PK parameters, as measured by the coefficient of variation, was generally moderate (~30–50%). There was no evidence of accumulation as concentrations measured prior to the last dose were minimal (7.7 – 63.3 ng/mL) and accumulation ratios for C_{max} and AUC were less than one.

Because similar PK values (geometric mean of the AUC 17.6 h*μg/mL and C_{max} was 5.0 μg/mL, respectively, and median half-life was 5.0 h) were reported in beagles (Rausch-Derra et al., 2016), when given 3X the dose of the same formulation the collies received, a population pharmacokinetic analysis was performed utilizing data from the current study combined with the data from the beagles in this reference. Both body weight and breed had significant impact on the model fit as covariates. Body weight and breed were highly correlated, where all beagle dogs weighed (range: 7.9–10.3 kg) much less than all collies (range: 17.2 – 32.8 kg). Because of the lack of data measured at intermediate body weights and because of the known physiological/pharmacological differences caused by lack of P-gp function, the final model was chosen with breed as the covariate. Final parameter estimates are provided in Table 4. Volume of the peripheral compartment of collies was estimated to be 45% that of beagles, and clearance from the central compartment was 71% less in collies than beagles.

4 | DISCUSSION/CONCLUSION

In this study, collie dogs homozygous for MDR1-1Δ were administered either grapiprant at a dosage of approximately 2 mg/kg or placebo once daily for 28 days. Grapiprant was rapidly absorbed with a T_{max} of approximately 1 h and eliminated with a half-life of approximately 5 h. Based on this half-life, accumulation of grapiprant would be expected to be minimal, which was confirmed by C_{max} and AUC accumulation ratios of less than 1. Although median T_{max} (1.0 h) and mean elimination $t_{½}$ (4.6 h) in collies homozygous for MDR1-1Δ agree with previously reported (Lebkowska-Wieruszewska et al., 2017) results in Labrador retrievers (i.e., 1.0 h and 5.2 h, respectively), the exposure does not. In the study, the C_{max} achieved in Labrador retrievers, presumed to have normal P-gp function, was 1.6 μg/mL and the AUC estimated to infinity was 4.8 h*μg/mL. By comparison, the C_{max} was 5.2 μg/mL and AUC estimated to infinity was 17.6 h*μg/mL when the same dose of grapiprant was administered to collies homozygous for MDR1-1Δ. Thus, total exposure to grapiprant is substantially higher. The exposure, represented by C_{max} and AUC, achieved in the collies after treatment with approximately 2 mg/kg of the tablet formulation (5.2 μg/mL and 17.6 h*μg/mL) exceeded that of beagles treated with 6 mg/kg (of a suspension in the nine-month toxicity study (3.7 μg/mL and 13.2 h*μg/mL) (Rausch-Derra et al., 2015) and with 3 mg/kg of a suspension (3.6 μg/mL and 7.6 h*μg/mL) (Nagahisa & Okumura, 2017).

There are several potential reasons for the greater total exposure of grapiprant in P-gp-deficient collies as compared to other breeds such as beagles and Labrador retrievers. P-gp deficiency could result in increased oral bioavailability since P-gp is expressed on enterocytes. However, several other P-gp substrates did not have increased oral bioavailability in collies homozygous for MDR1-1Δ compared to wild-type collies (Mealey et al., 2010). Decreased biliary excretion may be the most likely explanation for enhanced total exposure of grapiprant in P-gp-deficient collies as compared to beagles and Labrador retrievers. Biliary excretion has been shown to be essentially absent in collies homozygous for the MDR1 −1Δ (Coelho et al. 2009), and since more than 50% of grapiprant is excreted in the bile (EMA EPAR, 2018), curtailed biliary excretion would certainly enhance total exposure. It is of note that flattening of the elimination phase of the concentration time curve in beagles, which might represent enterohepatic circulation (see Figure 1, Rausch-Derra et al., 2016), was absent in collies (Figure 1). Although disparate P-gp function seems the most apparent reason for differences in total exposure in collies as compared to beagles, there could be other differences between these breeds that could account for the pharmacokinetic

differences. Another polymorphism that has been described in beagles is paired single nucleotide polymorphisms in the albumin gene that alter its amino acid sequence. Beagles homozygous for this polymorphism have altered plasma protein binding for some highly protein bound drugs (Mackin et al., 2020). Since grapiprant is over 95% protein bound, it is possible that this polymorphism contributes to the apparent breed differences in grapiprant disposition.

The occurrence of transient, self-limiting vomiting, and mild hypoalbuminemia and hypoproteinemia in the current study were not unexpected, having been identified in a grapiprant toxicity study in beagles administered dosages of 1, 6, and 50 mg/kg using a developmental liquid suspension formulation (Rausch-Derra et al., 2015). Mild signs of gastrointestinal disturbance, such as occasional vomiting and soft or mucoid feces that occasionally contained blood, were observed in the treated and control groups but to a greater extent in the highest dose group (50 mg/kg). Decreases in serum total protein and/or albumin concentrations were attributed to grapiprant administration. Several beagles treated with grapiprant had serum total protein and albumin concentrations that were less than respective lower reference limits, but these values were only mildly low and were not associated with clinical signs. These changes were statistically significant only in the highest dose group. Because of the greater exposure in collies compared to MDR1 wild-type dogs, it is not surprising that a tendency toward decreased serum albumin and total protein was seen in the collies. Each, along with vomiting, diarrhea, and decreased appetite are included as potential adverse reactions on the label.

One adverse effect that was *not* observed was central nervous system (CNS) toxicity. This is considered to be the "classical" toxicity of dogs with MDR1-1Δ and is, indeed, associated with many P-gp substrate drugs. For some of these drugs, toxicity is associated with increased ability to penetrate the blood–brain barrier, without achieving higher systemic blood concentrations. Other P-gp substrate drugs do not cause CNS toxicity in dogs with MDR1-1Δ, but these dogs are more sensitive to the adverse effects that are typically associated with the drug such as bone marrow suppression (vincristine) (Mealey, Fidel, et al., 2008) or immunosuppression (cyclosporine A) (Mackin et al., 2020). For these P-gp substrate drugs, increased systemic exposure at lower doses is more likely to cause adverse events in dogs with MDR1-1Δ than in MDR1 wild-type dogs. This appears to be true with grapiprant, where at the labeled dosage, transient vomiting and/or mild hypoalbuminemia seems to be more likely in dogs homozygous for MDR1-1Δ than in wild-type dogs, although the limited number of animals treated with grapiprant in this study make definitive conclusions problematic.

From a clinical perspective, the key question is "Does grapiprant dosing need to be adjusted for dogs with MDR1-1Δ as is the case for other drugs that are P-gp substrates?" While this was beyond the scope of the present study, it seems prudent to propose some reasonable approaches. As has been recommended for other P-gp substrate drugs, a 25% to 50% dose reduction could be made for dogs heterozygous or homozygous, respectively, for MDR1-1Δ. The dose could then be increased as tolerated to the full 2 mg/kg dose. Alternatively, for dogs with more severe pain and/or inflammation, the full dose could be administered as long as the pet owner is able to carefully monitor their dog for adverse events and return in 4–6 weeks for evaluation of plasma protein concentration.

In conclusion, this is the first study to examine the pharmacokinetics and tolerability of a widely used anti-inflammatory drug in dogs homozygous for MDR1-1Δ. Because canine genetic testing is widely available, many dog owners are aware of their dog's MDR1 genotype. Owners of dogs that harbor MDR1-1Δ have a strong desire to treat that animal only with drugs that have been tested for safety in these dogs (personal experience, KLM). This study generated key information regarding the disposition and safety of grapiprant in those dogs.

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CONFLICT OF INTEREST

Katrina L. Mealey discloses a consulting relationship with Elanco Animal Health for which she receives remuneration.

DATA AVAILABILITY STATEMENT

Research data are proprietary and are not shared.

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